INTRODUCTION

Renal transplantation is an established therapy for the treatment of end-stage renal disease. For patients with end-stage renal disease, their kidneys no longer work well enough to remove wastes and excess fluids from the body. These excess wastes and fluids build up in the blood and affect the whole body. Therefore, patients with end-stage renal disease must undergo the renal replacement therapy to stay alive. Renal replacement therapy, a term describing the various substitution treatment available for severe acute and end-stage chronic renal disease, contains dialysis and renal transplantation. Dialysis, both peritoneal dialysis and hemodialysis, is a therapy to remove excess wastes and fluids by diffusion of solutes across a membrane down a concentration gradient. This is the temporary therapy that patients with end-stage renal disease need to take every week. However, some patients with end-stage renal disease who suffer with the disadvantages of therapy, including the high cost and high complications, have to stop this therapy. Another therapy for patients with end-stage renal disease is the renal transplantation. This therapy offers the greatest potential for restoring a healthy life, lower cost, minimal complications with the better quality of life. However, renal transplantation is the transplantation of a kidney from a compatible donor to restore kidney function in a recipient. Even though the compatibility has been passed, there is always the episode that is called "rejection". Rejection is causing from a transplant recipient's immune system attacks a transplanted kidney. This episode causes decrease in renal blood flow and renal function, finally causing graft loss. Rejection divides into 2 parts, one is acute rejection and the another one is chronic rejection[1-4].

Acute rejection occurs greatest in the first 3 months, less in the next 6 months and relatively uncommon 1 year following transplantation. Acute rejection is characterized by a rapid decrease in renal function and blood flow, which is reflected by a fall in urine output and glomerular filtration. Clinically the most common signs are fever, graft enlargement, and tenderness, but this rejection may also be a subtle clinical process evidenced only by the biochemical indices of

decreased glomerular filtration. Note that as the transplant kidney cannot have any intact sensory nerves, pain at the site of the allograft duing acute rejection must result from swelling and inflammation of surrounding host tissues[3].

Late or chronic rejection refers to a rejection episode, occurring approximately 6 months or more after transplantation. It is often an insidious process, manifesting as a slow, inexorable rise in serum creatinine, fluid retention, peripheral edema, and hypertension. Pathologically, it is characterized by progressive damage to the transplant arterial intima resulting in fibrosis and luminal obliteration. This process results in irreversible tissue ischemia as evidenced by interstitial fibrosis and glomerular sclerosis[3,5].

Thus, taking the immunosuppressive agents after successful renal transplantation is important in order to prevent and treat the rejection episode. Immunosuppressive agents consist of many kinds of drugs, which act on the body's immune system with different mechanisms of action. Two of the widespread used immunosuppressive agents are "calcineurin inhibitors, cyclosporine and tacrolimus". Both cyclosporine and tacrolimus have potent immunosuppressive activity and selective ability to inhibit early T-cell calcium-dependent signaling events following T-cell receptor triggering. These inhibitory effects result in inhibition of expression of several cytokine genes, including interleukin (IL)-2, IL-3, IL-4, interferon-gamma, and tumor necrosis factor-gamma. Their indications are for preventing allograft rejection and graft-versus-host disease. Allograft survival rates for renal transplant recipients have significantly improved since these drugs were introduced for clinical use. Although the relationship has been demonstrated between blood or serum concentration and therapeutic effect, the difference between subtherapeutic and toxic concentrations of drugs are narrow. In addition, dosing of drugs are complicated by unpredictable, large intra- and interindividual variability in their pharmacokinetics. Many factors influence the pharmacokinetics of these agents. The therapeutic effect of cyclosporine and tacrolimus in organ transplantation cannot be readily assessed and their concentrations cannot be predicted on the basis of the dose alone. In considering the serious consequences of subtherapeutic drug concentrations, such as allograft rejection, and of toxic concentrations, such as renal failure, the careful monitoring of cyclosporine and tacrolimus concentrations are essential for optimal care of patients receiving these drugs[2,6,7].

Hyaluronic acid (HA) or hyaluronan, an ubiquitous polyanionic glycosaminoglycan predominantly derived from connective tissue ground substances, is a major constituent of the extracellular matrices which serves a variety of functions, including bone formation, cartilage structural maintenance, lubrication, wound healing, and interaction with the immune system. Besides, it also has strong water-binding capacity and thereby contributes to the cellular differentiation and migration. The HA level in human serum is constant because of the balance in biosynthesis and degradation. In adults aged 20-60 years, serum HA normally ranges from 10 to 100 μg/l. Markedly high level is noted in certain liver diseases, especially in patients with cirrhosis, when the clearance is impaired. Increased HA level is also seen in patients with rheumatoid arthritis, patients with other inflammatory diseases and certain tumors[8-10].

The previous study in 1987, Hallgren et al. found that serum HA levels are significantly increased in patients with renal insufficiency and with end-stage renal failure compared with the levels measured in age- and sex-matched healthy controls. Significant correlation is found between serum HA and degree of impaired renal function. None of the patients have laboratory signs indicating affection of the liver, the major elimination route for circulating HA[8]. In 1990, Hallgren and colleagues also found that in the normal rat kidney, HA is localized in the extracellular space of the inner medulla and increased markedly towards the papillary tip. No staining for HA is seen in the interstitial tissue of the cortex or the outer medulla. During the development of rejection of allogeneic renal grafts, a progressive increase in accumulated HA is seen in the interstitial tissue of the cortex and outer medulla. The relative water content of the cortex and outer medulla also increases progressively and correlates with the HA accumulation[11], which is similar to the study of Wells et al. He and his colleagues reported the localization of HA in normal, acutely rejecting and chronic rejecting human kidneys. In the normal kidneys, HA is essentially confined to the medulla, but those acute rejection kidneys have the increase of HA both in the cortex and in the medulla. Similar to the chronic rejecting kidneys, increased HA amounts are observed primarily in the cortex and in sclerotic vessels. When incubating tissue sections with hyaluronidase abolishes the staining for HA[12-13]. In 1999, Stenvinkel et al. demonstrated that the serum levels of HA are markedly elevated in patients with chronic renal failure. In contrast, the HA levels after the successful renal transplantation have been shown to return to normal[14]. In addition, Johnssons et al. found that the increased tissue content of water

and HA seen during allograft rejection can significantly reduce by treatment with the HA-degrading enzyme hyaluronidase alone or in combination with cyclosporine. Morphological examination reveals grafts with better-preserved morphology and fewer infiltrating mononuclear cells compared with untreated controls[15]. Moreover, Knoflach et al. demonstrated HA, in soluble form, can inhibit interactions between T-lymphocyte CD44, homing receptor of HA, and HA *in vivo* in the models of acute and chronic allograft rejection. In acute rejection model, combined cyclosporine plus low molecular weight HA therapy can prevent acute rejection and significantly prolong graft survival. This group also demonstrates better preservation of transplant organ. Histological graft analysis of combined treated animals show minimal rejection and leukocyte infiltration, compare with other groups. Intragraft gene expression analysis, using semiquantitative reverse transcription-polymerase chain reaction, the combined-treatment group show reductions of CD4, CD8 and interferon-gamma transcript levels. In chronic rejection model, they showed that the transplant animals treated with cyclosporine plus low molecular weight HA significantly increase long-term graft survival, decrease mononuclear cell infiltration, and afford better preservation of myocardial architecture compared with cyclosporine-treated group. In addition, this therapy exhibits decreased expression of interferon-gamma, the growth factors transforming growth factor-beta, platelet-derived growth factor and fibrogenic growth factor-beta[16-17]. In 2000, Zhang et al. evaluated the treatment of rat renal and cardiac allograft recipients with low molecular weight HA. They found that low molecular weight HA monotherapy significantly prolongs allograft survival, but only for a few days. In combination with low-dose cyclosporine, long-term survival of allografts is observed in some recipients[18]. In 2002, Hellkvist et al. demonstrated that the isolate fibroblasts from rejecting tissue display strongly up-regulated HA production and proliferation rate as compared with fibroblasts from normal tissue. In addition, they also found several cytokines are involved in the regulation of fibroblast activity[19].

From the relationship between increased HA levels and the rejection episode after transplantation, including the immunosuppressive drug level monitoring in renal transplant patients, the purpose of this study is to evaluate the correlation of HA level and immunosuppressive drug levels in renal transplant patients. In addition, from this study may also imply the level of HA to be a biochemical marker for prognosis the rejection episode.