

Relationship between changes of calcineurin inhibitor levels and serum creatinine and cystatin C levels in kidney transplant patients

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- Background** : Calcineurin inhibitors (CNI: cyclosporine, tacrolimus) are major immunosuppressants used in kidney transplant (KT) patients. Nephrotoxicity is an important side effect of CNI. The elevation of serum creatinine (Scr) is a biomarker for nephrotoxicity. However, there are several factors that affect changes of Scr. It is believed that serum cystatin C (Scys C) would be a better biomarker.
- Objective** : To investigate the relationship between changes of CNI levels and Scr and Scys C levels in KT patients.
- Design** : Descriptive study
- Setting** : Division of Nephrology, Department of Medicine, Phramongkutklao Hospital
- Patients and Methods** : Patients who received KT for at least 3 months, and during steady state. Blood samples were collected for determining cyclosporine and tacrolimus trough levels, Scr and Scys C levels.

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- Results** : 217 serum samples were analysed from 56 patients (43 male, 13 female). The results showed that the changes of CNI levels were not correlated with the changes of Scr ($r = -0.103$, $P = 0.194$) and Scys C ($r = -0.107$, $P = 0.176$) levels.
- Conclusions** : Patients who received KT for a long time until the steady state, the slightly changes of CNI levels, no influence on the changes of Scr and Scys C levels. Therefore, biomarker can be used both Scr and Scys C
- Keywords** : Calcineurin inhibitors, creatinine, cystatin C, kidney transplant.

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- เหตุผลของการทำวิจัย** : ยา cyclosporine และ tacrolimus เป็นหนึ่งในยากดภูมิคุ้มกันที่สำคัญที่ใช้ในผู้ป่วยปลูกถ่ายไต แต่ยานี้มีผลข้างเคียงที่สำคัญคือความเป็นพิษต่อไต ซึ่งปกติวัดโดยการเพิ่มขึ้นของระดับครีเอตินินในซีรัม อย่างไรก็ตามยังมีปัจจัยหลายประการที่มีผลต่อการเปลี่ยนแปลงของระดับครีเอตินินในซีรัม ซึ่งเชื่อว่าระดับซิสแททิน ซี ในซีรัมน่าจะเป็นตัวชี้วัดที่ดีกว่า
- วัตถุประสงค์** : หาความสัมพันธ์ระหว่างการเปลี่ยนแปลงของระดับยา cyclosporine และ tacrolimus ในเลือดกับการเปลี่ยนแปลงของระดับครีเอตินินและซิสแททิน ซี ในซีรัมของผู้ป่วยปลูกถ่ายไต
- รูปแบบการวิจัย** : การศึกษาเชิงพรรณนา
- สถานที่ทำการศึกษา** : แผนกโรคไต กองอายุรกรรม โรงพยาบาลพระมงกุฎเกล้า
- ตัวอย่างและวิธีการศึกษา** : ผู้ป่วยที่ปลูกถ่ายไตมานานอย่างน้อย 3 เดือน และอยู่ในสภาวะคงที่ ผู้ป่วยจะถูกเจาะเลือดก่อนการให้ยาครั้งต่อไปเพื่อตรวจระดับ cyclosporine และ tacrolimus ในเลือด, ระดับครีเอตินินและซิสแททิน ซี ในซีรัม
- ผลการศึกษา** : ได้ตัวอย่างซีรัมจำนวน 217 ตัวอย่าง จากผู้ป่วย 56 ราย (เพศชาย 43 ราย, เพศหญิง 13 ราย) จากผลแสดงให้เห็นว่าการเปลี่ยนแปลงของระดับยา cyclosporine และ tacrolimus ในเลือดไม่มีความสัมพันธ์กับการเปลี่ยนแปลงของระดับครีเอตินิน ($r = -0.103, P = 0.194$) และซิสแททิน ซี ($r = -0.107, P = 0.176$) ในซีรัม
- สรุป** : ผู้ป่วยที่มีการปลูกถ่ายไตมานานจนอยู่ในสภาวะคงที่แล้ว การเปลี่ยนแปลงของระดับยา cyclosporine และ tacrolimus ในเลือดเพียงเล็กน้อยไม่มีผลต่อการเปลี่ยนแปลงของระดับครีเอตินินและซิสแททิน ซี ในซีรัม ดังนั้น ตัวชี้วัดสามารถใช้ได้ทั้งครีเอตินินและซิสแททิน ซี ในซีรัม
- คำสำคัญ** : ยา cyclosporine และ tacrolimus, ครีเอตินิน, ซิสแททิน ซี, ปลูกถ่ายไต

In kidney transplant patients, the standard immunosuppressive regimen consisted of corticosteroid, calcineurin inhibitors (CNI: cyclosporine, tacrolimus), often combined with azathioprine or mycophenolate or mTOR, now used as an adjunctive agent in so-called triple therapy.⁽¹⁾ Nephrotoxicity is an important side effect of CNI. Because the drugs causes renal vasoconstriction.⁽²⁾ This is the one mechanism that causes acute kidney injury (AKI).⁽²⁾

AKI represents a major clinical problem, with rising incidence and high mortality rate.^(3,4) Over the past 50 years, mortality rates of patients with AKI in intensive care unit (ICU) approximate 50% to 70%.⁽⁴⁾ Incidence based on population studies such as 4% to 15% of patients after cardiopulmonary bypass and could rise to 19% to 51% in critically ill patients.⁽⁵⁾

Criteria of Acute Kidney Injury Network (AKIN) classification system⁽⁶⁾, the diagnosis of AKI is based on either the elevation of serum creatinine (Scr) or the reduction in urine output.^(3,4) However, Scr is a poor biomarker.^(3,4) Because of numerous factors influencing the Scr concentration, such as body weight, race, age, sex, drugs, muscle mass, and protein intake.⁽³⁾ Therefore, current interest in the new biomarkers replace Scr, such as Neutrophil Gelatinase-Associated Lipocalin (NGAL) in blood and urine⁽³⁾, Interleukin-18 (IL-18) in urine⁽³⁾, Kidney Injury Molecule-1 (KIM-1) in urine⁽³⁾, and cystatin C in blood and urine.⁽³⁾

Cystatin C is produced at a constant rate by all nucleated cells, free filtration at the glomerulus, complete reabsorption and catabolism by the proximal tubule cells, and lack of effect of sex, age, race and muscle mass.^(3,4,7) Multiple studies have

been performed to evaluate the accuracy of serum cystatin C (Scys C) level as a marker of glomerular filtration rate (GFR). Most of these studies have shown that Scys C level is a significantly better marker of GFR than Scr based on analysis of correlation coefficients.⁽⁸⁻¹²⁾ Therefore, Scys C may represent a biomarker for AKI better than Scr.

To compare the sensitivity of Scys C and Scr in relation to CNI nephrotoxicity in kidney transplant patients. Therefore, the purpose of the study is to investigate the relationship between changes of CNI levels and Scr and Scys C levels in kidney transplant patients.

Patients and Methods

Study population

The study design was descriptive with was done from November 2009 to May 2010. Inclusion criteria were patients who received kidney transplants for at least 3 months, and during steady state conditions (not occur graft rejection within the past 3 months and > 1 month of treatment with stable dose of CNI). Exclusion criteria were patients with thyroid disease, severe infection, and receiving corticosteroid more than 1 mg/kg/day. This project has been approved by Ethics Committee of Royal Thai Army medical Department.

Operative definition

Changes of CNI, Scr and Scys C levels were defined as percentage of the difference of CNI, Scr and Scys C levels. For example, patients had a follow up 4 times, we collected 4 values of each the CNI, Scr and Scys C levels and 3 values of each the changes of CNI, Scr and Scys C levels.

Data collection

All included patient were reviewed baseline data from medical records. Blood samples were collected before routine follow up for determining cyclosporine and tacrolimus trough levels, Scr and Scys C levels. Scr was determined using a enzymatic colorimetric method.⁽¹³⁾ Scys C was determined using a particle-enhanced nephelometric immunoassay (PENIA).⁽⁷⁾ Cyclosporine level was determined using fluorescence polarization immunoassay (FPIA).⁽¹⁴⁾ Tacrolimus level was determined using microparticle enzyme immunoassay (MEIA).⁽¹⁴⁾ Normal Scr values range from 0.77 – 1.34 mg/dL⁽¹⁵⁾, and normal Scys C values range from 0.51 – 0.98 mg/L.⁽¹⁵⁾ Therapeutic level for cyclosporine about 150 ng/mL.⁽¹⁾ Therapeutic level for tacrolimus range from 5 – 15 ng/dL.⁽¹⁾

Statistical analysis

The data is expressed in mean \pm standard deviation (range). Correlations between changes of CNI levels and Scr and Scys C levels were determined

using Pearson's test. Correlations between Scr and Scys C levels were determined using Pearson's test. Significance was defined as a $P < 0.05$. For statistical analysis, the SPSS version 17.0 program was used.

Results

The clinical characteristics of the patients are shown in Table 1 and 2. 217 serum samples were analysed from 56 patients (43 male and 13 female) who had undergone kidney transplant, with a mean age 47.6 ± 9.3 years (range 23 - 64 years). All patients were receiving oral prednisolone and CNI (33 cyclosporine and 23 tacrolimus). Prednisolone dosages were adjusted from -5.00 to -0.71 mg. From 217 serum samples, there were 161 values of the changes of CNI levels and Scr and Scys C levels. The changes of CNI levels were not correlated with the changes of Scr ($r = -0.103$, $P = 0.194$) and Scys C ($r = -0.107$, $P = 0.176$) levels (Fig. 1 and Fig. 2). We found a highly significant correlation between Scr and Scys C levels ($r = 0.887$, $P < 0.01$) (Fig. 3).

Table 1. Characteristics of patients.

Parameter	Kidney transplants patients (n = 56)
Sex (male / female)	43 / 13
Age (year)	47.6 ± 9.3 (23 – 64)
Weight (kg)	64.8 ± 12.1 (40 – 104)
Height (cm)	165.5 ± 7.9 (140 – 180)
Kidney transplant duration (month)	101.7 ± 64.4 (3 – 247)
CDKT / LRKT	42 / 14
Hypertension / Hyperlipidemia / Diabetes	50 / 47 / 7
Number of immunosuppressive agents (2 groups / 3 groups / 4 groups)	5 / 50 / 1

CDKT, Cadaveric donor kidney transplant; LRKT, Living related donor kidney transplant

Table 2. Biochemical parameters of patients (n = 217).

Parameter	Mean ± SD (range)
Cyclosporine level (ng/mL)	102.30 ± 40.65 (17.40 – 232.40)
Δ Cyclosporine level (%)	10.36 ± 58.20 (-68.19 – 266.00)
Tacrolimus level (ng/dL)	6.17 ± 1.98 (1.90 – 12.60)
Δ Tacrolimus level (%)	3.03 ± 35.83 (-53.66 – 108.57)
Serum creatinine (mg/dL)	1.54 ± 0.66 (0.60 – 5.00)
Δ Serum creatinine (%)	1.13 ± 10.81 (-35.71 – 38.89)
Serum cystatin C (mg/L)	1.43 ± 0.60 (0.78 – 4.94)
Δ Serum cystatin C (%)	3.24 ± 19.99 (-49.62 – 77.23)
Blood urea nitrogen (mg/dL)	20.16 ± 8.24 (8.50 – 62.00)
GFR: CG (ml/min/1.73 m ²)	53.09 ± 15.36 (15.51 – 83.78)
CKD-EPI (ml/min/1.73 m ²)	57.44 ± 19.03 (12.22 – 105.54)

CG, Cockcroft-Gault; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration

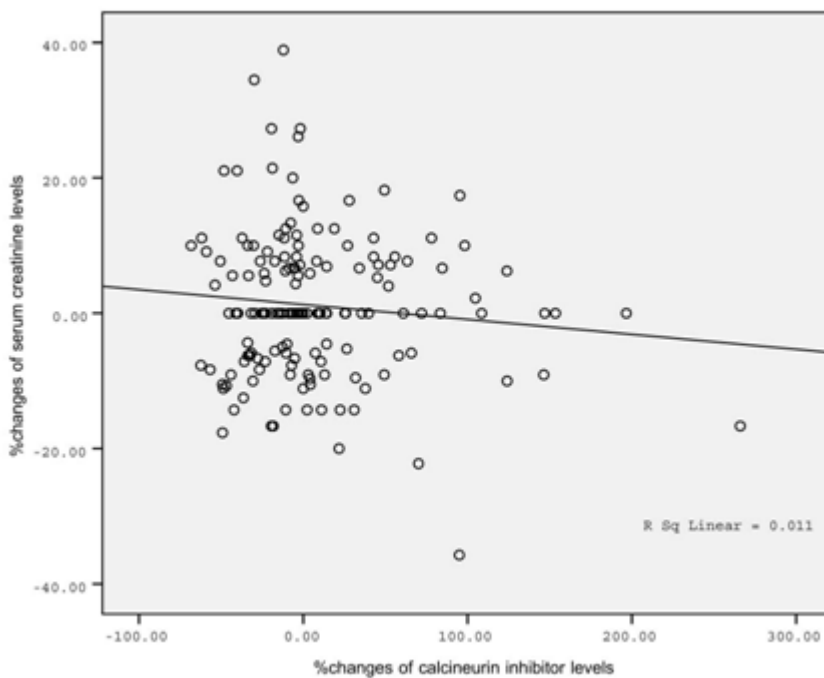


Figure 1. Relationship between changes of serum creatinine (r = -0.103, P = 0.194) levels and changes of calcineurin inhibitor levels.

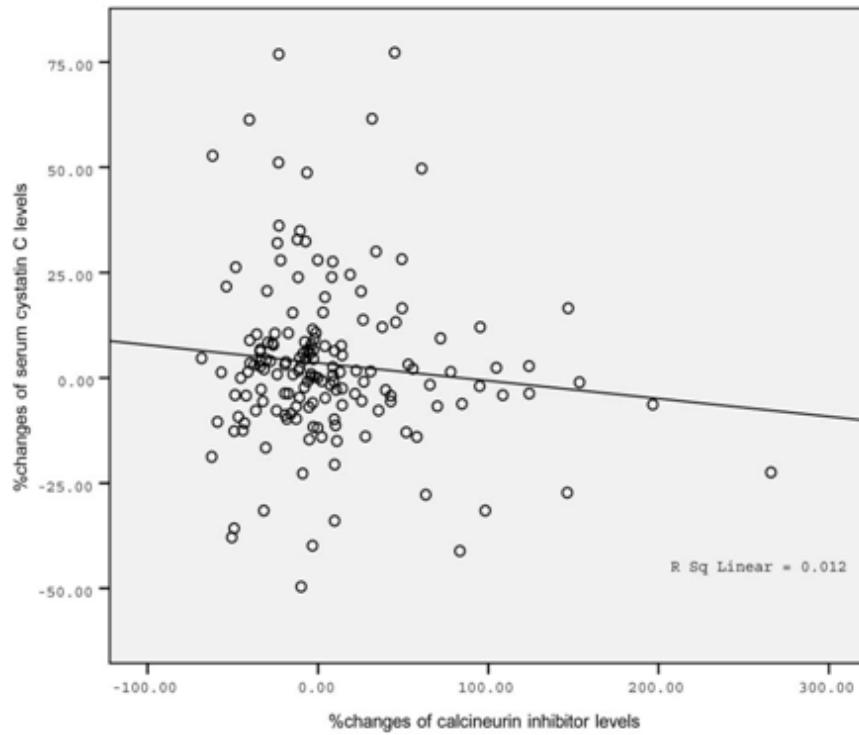


Figure 2. Relationship between changes of serum cystatin C ($r = -0.107$, $P = 0.176$) levels and changes of calcineurin inhibitor levels.

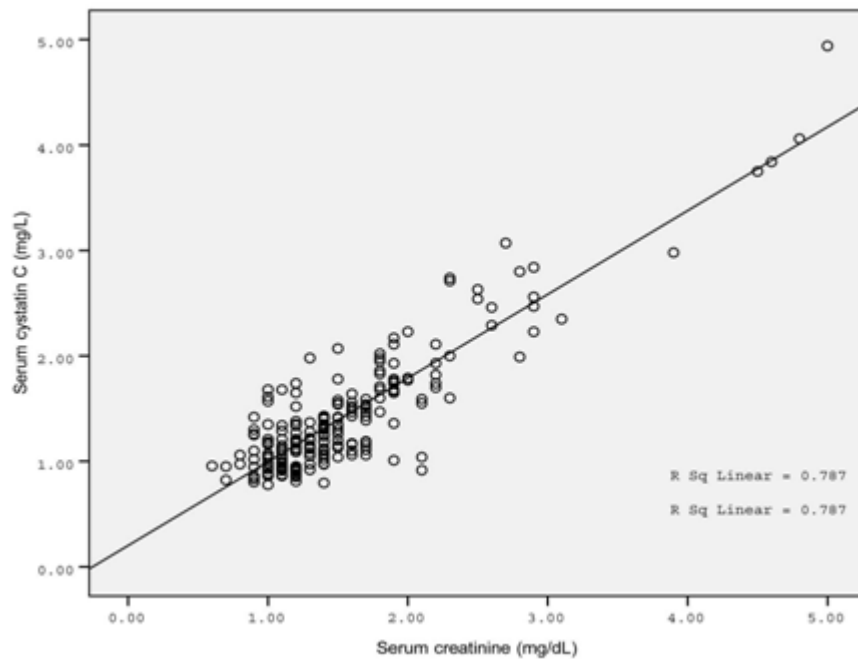


Figure 3. Relationship between of serum creatinine and cystatin C levels ($r = 0.887$, $P < 0.01$).

The changes of Scr levels $\geq 50\%$ was not found, whereas the changes of Scys C levels $\geq 50\%$ were found 6 times in 161 times (3.7%) In this group, the changes of CNI levels tended to correlate with the changes of Scys C ($r = 0.771$ $P = 0.072$) levels, whereas the changes of CNI levels were not correlated with the changes of Scr ($r = -0.543$ $P = 0.266$).

Discussion

No previous study is to investigate the relationship between changes of CNI levels and Scr and Scys C levels in kidney transplant patients. In this study, we found the changes of calcineurin inhibitor levels were not correlated with the changes of Scr and Scys C levels. Results show that no correlation may be due to no patients with nephrotoxicity caused by CNI or physician adjusted CNI dosages from CNI levels, whereas Scr and Scys C levels were not increased. The changes of Scr levels $\geq 50\%$ defined as AKI⁽⁶⁾. In this study, the changes of Scr levels $\geq 50\%$ was not found whereas the changes of Scys C levels $\geq 50\%$ were found (3.7%). There was not a definition of AKI is based on the elevation of Scys C level. For patients who had Scys C levels $\geq 50\%$, the changes of CNI levels tended to correlate with the changes of Scys C levels. Therefore Scys C seems to be more sensitive biomarker than Scr for defined as nephrotoxicity.

Intra- and inter- individual variations of CNI, that may influence the immune response and susceptibility to drug toxicity.^(1,16,17) Results show that no difference in correlation between changes of CNI levels and Scr and Scys C levels may be due to Scr levels were highly correlated with Scys C levels, which is the same as the previous study.^(18,19)

Following cessation of concomitant prednisolone therapy, CNI levels may increase by up to 25% and Scr levels may increase.^(1,20) In this study, no patients with cessation of prednisolone. But, decreasing in prednisolone dosage might increase the CNI levels and Scr levels. Most patients (90.9%) had dyslipidemia which could reduce cyclosporine levels.^(21,22) Because cyclosporine is a lipophilic drug.^(1,23)

The study had limitation in follow up period, lab variation and the real trough level of CNI. Further study in drug induced nephrotoxicity should be studying in patients who received kidney transplant during the first period because patients chance more nephrotoxicity than patients who received kidney transplant for a long time.

In conclusion, patients who received kidney transplant for a long time until the steady state, the slightly changes of CNI levels, no influence on the changes of Scr and Scys C levels. In clinical practice, Scr level is commonly used as biomarker for defined as nephrotoxicity which were highly correlated with Scys C level. Therefore, monitoring of renal function in kidney transplant patients can be used both Scr and Scys C. However, Scys C can be used in patient with decreased muscle mass or using drug that influence Scr levels.

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