

Original Article

Serum Cystatin C can detect impaired graft function early after renal transplantation

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Received June 15, 2015; Accepted September 6, 2015; Epub September 15, 2015; Published September 30, 2015

Abstract: Objectives: To study the significance of Cystatin C (Cys C) in early detection of the graft function after renal transplantation. Material: The concentrations of Cys C, Blood urea nitrogen (BUN), Serum creatinine (SCr), and glomerular filtration rate (GFR) were measured. According to the results of the post-transplantation GFR; Seventy renal post-transplanted patients were assigned into 3 groups: group A (31 cases with the normal range of the renal function, $GFR \geq 90$ ml/min/1.73 m²), group B (27 cases with $Scr < 133$ μ mol/L and $60 < GFR < 90$ ml/min/1.73 m²), and group C (12 cases with $Scr \geq 133$ μ mol/L and $60 < Ccr < 90$ ml/min/1.73 m²). Meanwhile, 60 cases with normal renal function were applied as control group. Results: The Cys C concentration in group B (1.37 ± 0.14) was significantly higher than it in group A (0.96 ± 0.10) ($P < 0.01$). The SCr and the Cys C demonstrated linear correlations with the GFR in all groups of A, B and C. The correlation coefficients (r) in each group were -0.7272 and -0.7439, -0.7072 and -0.7543, -0.7430 and -0.7669; respectively. Conclusions: Compared with Scr, Cys C has better correlations with GFR when the graft function was normal or renal function was slightly impaired. Cys C could be used in the early detection of renal function impairment after transplantation.

Keywords: Cystatin C, graft, renal function, renal transplantation

Introduction

Serum creatinine (Scr) level is commonly used to estimate renal function, however, Scr is affected by not only its renal excretion, but also its production mainly from muscular tissue [1]. Scr is also highly dependent on age, weight, and gender. It leads to adjust the Scr value by these factors for estimating renal function, especially in renal transplant recipients. Rapid and accurate evaluation of renal function from glomerular filtration rate (GFR) is important in follow-up of renal transplant patients for detecting rejection and adapting drug dosage.

Cystatin C (Cys C), a cationic basic protein [2], is a cysteine proteinase inhibitor [3] and involves in intracellular protein catabolism [4]. It has a constant producing rate by all nucleated cells before being freely filtered at the glomeruli. Then Cys C is almost completely reabsorbed and catabolized in the proximal tubular cells [3, 5]. Cys C has therefore been evaluated as an endogenous marker for GFR in patients with various renal diseases and the different

stages of renal failure [3]. However, it is not yet to know if Cys C can detect early impairment of graft function after renal transplantation.

In this study, we tested if the Cys C is correlated with the GFR. The Modification of Diet in Renal Disease (MDRD) equation calculating the value of the GFR. The MDRD equation for classification of chronic kidney disease was supposed as an excellent marker for GFR [6, 7].

Methods

Patients

All patients have written-format informed consent with confidentiality. The study was approved by the institutional ethical committee. Data were collected from 70 renal transplant patients (Mean age was 40.4 ± 11.9 years. Sex ratio (male/female) was 44/26) during hospitalization from August 2009 to April 2012.

The donor kidneys were from relatives (n=35, aged between 20-55 years old, healthy, the ratio male to female 1:2) or from brain death

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Table 1. The value of Scr, GFR and Cys C (mean ± SD) in three groups

Groups	Cases (n)	Scr (µmol/L)	Cys C (mg/L)	GFR (ml/min/1.73 m ²)
A	31	90.51±9.86	0.96±0.10**	101.37±9.47
B	27	105.26±13.76	1.37±0.14*	80.99±5.20
C	12	188.98±18.84	2.77±0.31	64.97±4.20
Normal control	60	50.48±5.20	0.72±0.13	102.45±18.56

*: $t=8.9878$, $P=0.0000<0.01$, vs group A, T-test. **: $t=12.5636$, $P=0.0000<0.01$, vs control, T-test.

patients (n=35, aged between 18-50 years old, the ratio male to female 2:1). No interest or trading existed between the donors and recipients.

Collection of the peripheral blood samples

The blood samples were obtained during hospitalization. A 2 ml blood sample was taken before operation or the immune suppressant given, Peripheral blood sample was taken every day within a week post-transplant. Then the blood sample was taken every week during three months after transplantation.

According to Daniel et al [8] defining renal failure, the cut point of GFR value was 90 mL/min. Two cut-off values of inulin clearance were studied: 60 and 90 mL/min/1.73 m². GFR was determined by the MDRD formula. According to the value of GFR post-transplantation, 70 cases of renal transplant patients were divided into group A (31 cases, with the normal renal function, $GFR \geq 90$ ml/min/1.73 m²), group B ($Scr < 133$ µmol/L, $60 < GFR < 90$ ml/min/1.73 m², 27 cases), and group C ($Scr \geq 133$ µmol/L, $60 < Ccr < 90$ ml/min/1.73 m², 12 cases). Group B and C were called the lighter renal function damage group. Meanwhile, the normal control group (n=60) was compared.

Immunosuppressive treatment at the time of the evaluation included: cyclosporin A twice daily (7 mg/kg/d), glucocorticoids, and either mycophenolate mofetil (1.5 g/d) or azathioprine.

Laboratory methods

All blood work was drawn between 8 and 9 am without having taken the morning medication. Scr was determined by use of an enzymatic

method (Kodak Ektachem 700 XR-C system using the enzymes creatinine amidohydrolase and creatinine amidohydrolase). Total analytical (intra-assay + inter-assay) imprecision of the method was 2.6% (using a control sample with a concentration of 80 µmol/l). Reference range: 55-132 µmol/l (men), 45-120 µmol/l (women) [9].

We referred the renal function as normal, when Scr was under 133 µmol/l.

Determination of Cys C was performed by the commercially available N Latex Cystatin C kit (Dade Behring Canada Inc., Mississauga, Canada) on a Behring BN II Pro Spec analyzer (Dade Behring Canada Inc.). We also calculated the GFR based on Cys C using the formula described. Reference range: 0.6-1.20 mg/l (1-55 years).

Simultaneously, the GFR was calculated on the basis of the following formula⁽⁶⁾:

$$\begin{aligned} \text{MDRD} &= 186 \times \left[\text{serum creatinine (mg/dL)} \right]^{-1.154} \\ &\times \left[\text{age (years)} \right]^{-0.203} \\ &\times \left[0.742 \text{ if patient is female} \right] \\ &\times \left[1.21 \text{ if patient is African _ American} \right] \end{aligned}$$

Statistical analysis

All data are expressed by mean ± standard deviation. T-test and linear correlation analyses were used in comparison means of two groups. A statistical significance was determined at $P < 0.05$. Version 11.5 of the SPSS computer program (SPSS Institute, Chicago, USA) was used in the analysis.

Results

The results of Cys C, and Scr in different ranges of GFR collected in **Table 1**. The GFR cutting-off value defining renal failure was chosen at 90 mL/min/1.73 m². According to such criteria, $GFR < 90$ mL/min/1.73 m² was observed in 39 of the 70 samples. For subjects with normal GFR ($GFR \geq 90$ mL/min/1.73 m²); the mean Cys C was 0.96 ± 0.10 mg/L, the mean Scr was

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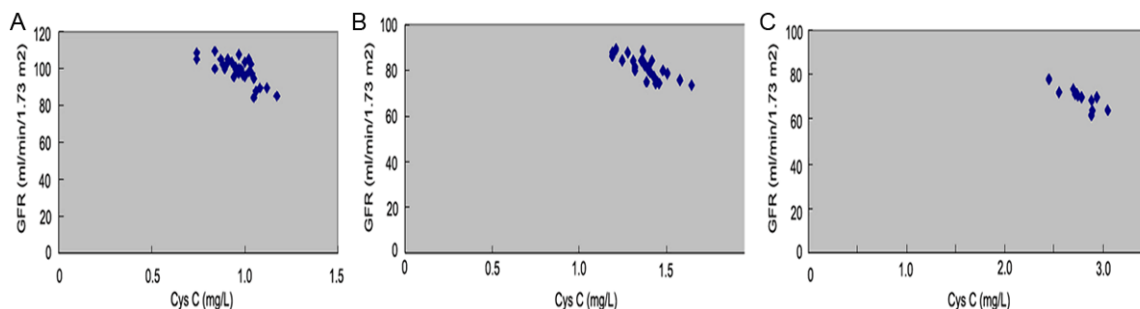


Figure 1. A. The correlation coefficient (r) was -0.7439 in group A for the relationship between Cys C (Cystatin C) and GFR (Glomerular Filtration Rate). The GFR was determined by the MDRD equation (Modification of Diet in Renal Disease equation). B. The correlation coefficient (r) of group B was -0.7543 between Cys C (Cystatin C) and GFR (Glomerular Filtration Rate). The GFR was determined by the MDRD equation (Modification of Diet in Renal Disease equation). C. The correlation coefficient (r) of group C was -0.7669 between Cys C (Cystatin C) and GFR (Glomerular Filtration Rate). The GFR was determined by the MDRD equation (Modification of Diet in Renal Disease equation).

90.51 ± 9.86 $\mu\text{mol/L}$, and the mean GFR was 101.37 ± 9.47 ml/min/1.73 m^2 . For patients with GFR at the range of 60 – 90 ml/min/1.73 m^2 , the mean Cys C were 1.37 ± 0.14 mg/L in group B and 2.77 ± 0.31 mg/L in group C, the mean Scr were 105.26 ± 13.76 $\mu\text{mol/L}$ in group B and 188.98 ± 18.84 $\mu\text{mol/L}$, and the mean GFR were 80.99 ± 5.20 mL/min/1.73 m^2 in group B and 64.97 ± 4.20 mL/min/1.73 m^2 in group C.

The correlation of Cys C with GFR was demonstrated significance (**Figure 1**). According to scatterplot, SCr and Cys C showed similar linear correlation with GFR in group A, group B and group C. The correlation coefficients (r) of each group were -0.7272 and -0.7439 , -0.7072 and -0.7543 , -0.7430 and -0.7669 , respectively.

Discussions

SCr is the most common biomarker for estimation of the kidney function. However, SCr is dependent on age, gender, and muscle mass [10, 11]. In addition, its tubular secretion is increased in the chronic renal failure. This limits the use of the SCr as a biomarker for estimating GFR, especially in those renal transplantation patients. To better estimate GFR, several low molecular weight proteins have been studied as potential biomarkers of the GFR. Cys C among them has become to be one of the most promising makers since the CysC concentration is independent of age when over 1 year old, height, weight, muscle mass, and gender [12, 13].

Like previous studies reported, [19–21] we found good correlations between Cys C and GFR; suggesting that Cys C is a precise and reliable index for renal function in renal transplant recipients. In our study, we demonstrated the concentration of the Cys C in group A had significant difference with normal control ($P < 0.01$, 0.96 ± 0.10 mg/l v.s. 0.72 ± 0.13 mg/l). After renal transplantation, the concentrations of the Scr and the BUN were back to normal range, while the concentration of the Cys C remained above the normal range. It is not yet to be explained exactly. Cys C has been demonstrated to be unaffected by several factors, such as gender and body weight [1, 14]. However, Cys C secretion by HeLa cells has been found to increase up to 80% following dexamethasone exposure in several *in-vitro* studies [15]. Therefore, other studies in clinic showed an increase of serum Cys C after high-dose corticosteroid application in renal transplantation patients [16, 17]. In contrast, the continuous low-dose corticosteroid treatment on Cys C response has remained in controversies. Moderate steroids therapy has not affect the level of the Cys C in pediatric nephrotic syndrome [18].

In our study, the stable transplant recipients ($\text{GFR} \geq 90$ ml/min/1.73 m^2) did not exhibit Cys C more rapid change than Scr according to correlated GFR. However, at the early stage of renal function impairment ($60 < \text{GFR} < 90$ ml/min/1.73 m^2), we demonstrated Cys C can help earlier diagnosis of renal function recovery than that of SCr, especially in patients with delayed graft function.

In our study, renal function impairment at the early stage as the GFR from 60 to 90 (ml/min/1.73 m²), was further divided into group B and group C according to normality or abnormality of the Scr concentration. In group B, the Cys C concentration was 1.37±1.04 mg/l (P=0.0000<0.01, compared to that of in group A as 0.96±0.10 mg/l). In Group B (total 27 cases), 8 cases occurred acute rejection at the early stage, 5 cases had a high Cyclosporine A serum level, 4 cases followed infections, and 10 cases had no documented clinical symptom. All these patients had lighter renal function impairment, while their concentrations of the Scr had not increased (Scr<133 μmol/l). Meanwhile, the Cys C in group C was 2.77±0.31 mg/l (P=0.0000<0.01, compared to that of in group A). In group C, 3 cases had a super-blood drug level, 5 cases were diagnosed as acute rejection and 4 cases followed delayed graft function (DGF).

The significant correlation of Cys C, Scr and GFR was demonstrated among GFR, Cys C, and Scr (**Figure 1**). From the scatterplots, SCr and Cys C showed similar linear correlation with GFR in group A, group B and group C. The correlation coefficient (r) of each group was -0.7272 and -0.7439, -0.7072 and -0.7543, -0.7430 and -0.7669, respectively. Compared to the Scr, the Cys C demonstrated better relevance with that GFR.

In conclusion, the Cys C concentration was not influenced by diet, infection and other metabolic status. The Cys C has a precise relevance with GFR at the early stage of renal function impairment. When GFR was below 90 ml/min/1.73 m², the Cys C demonstrated early changes. Therefore, Cys C, as a rapid and sensitive maker, can be used for the early diagnosis of renal function impairment after transplantation.

Acknowledgements

The authors thank Dr. Dianzheng Zhang (Philadelphia College of Osteopathic Medicine, Philadelphia, PA 19131) for dedicated review and discussions. Also thank Drs. Shuangde Liu and Xiaoli Liu (Department of Renal Transplantation, The Second Hospital of Shandong University) for their help in statistical analysis.

Disclosure of conflict of interest

None.

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