# Cystatin-C as an early marker of diabetic nephropathy in diabetic patients

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**Abstract**

Chronic kidney disease (CKD) is a major trouble in public health worldwide due to its rapid and random prevalence especially in diabetic patients. Further, diabetic nephropathy is a common cause of renal failure occurred in diabetic patients accompanied with low level of albumin in urine at early stage. Notably, early recognition of structural and functional alteration in the kidney is required and essential to identify the appropriate treatment at the beginning of renal syndromes. Several studies exhibit the essential role of serum creatinine in assessment of glomerular filtration rate (GFR) as a biomarker of renal failure. Alternatively, here findings demonstrate that level of serum cystatin-c is an efficacious candidate that can be revealing the possible alternations in renal function including children patients. Thus, blood samples collected from renal and/or diabetic patients showed contradictory relationship between serum cystatin-c level and GFR independent from diabetic syndromes. The results strongly indicate the possible exploiting of serum cystatin-c as an early indicator for renal disease. Collectively, current data further confirm the crucial role of serum cystatin-c in early detection of renal failure via reverse connection with GFR particularly in diabetic children.

## Introduction

Kidney disease or renal failure is a medical syndrome in which the kidney loses the ability to filter the waste from blood. Renal failure contains two major types, the acute kidney injury (AKI) and the chronic kidney disease (CKD). Usually, renal syndrome is determined by decrease in glomerular filtration rate (GFR) of waste production maintained by levels of creatinine in blood and blood urea nitrogen (BUN) (Hartemann *et al*., 2011; Liao et al., 2012; Lyman, 1986). Acute kidney injury normally arises when kidneys are overloaded with cytotoxic compounds or when blood supply is suddenly interrupted. Probably, drug overdoses, and chemotherapy, also cause the onset of acute kidney injury (Lieske et al., 2013). Unlike acute kidney injury, chronic kidney disease is caused by various medical conditions such as uncontrolled high blood pressure (hypertension), diabetes mellitus and polycystic kidney disease (Kes *et al*., 2011; Liao *et al*., 2012). Further, some

infectious disease such as Hantavirus can also attack the kidneys causing chronic kidney damage (Makela *et al*., 2000; Miettinen *et al*., 2006; Mustonen *et al*., 1996). Usually, hypertension and diabetes are the principal causes of chronic kidney disease mainly by affecting blood vessels causing structural and functional alternations in the kidneys. Together with hypertension, diabetic patients suffer from high glomerular pressure resulted in capillaries wall stretch, endothelial damage and glomerular protein secretion. Such syndrome is known as diabetic nephropathy or kimmelstiel-Wilson syndrome in which kidney glomeruli and capillaries formed angiopathy vessels leading to renal failure disorder (Hartemann *et al*., 2011; Kes *et al*., 2011). Notably, early recognition of glomerular nephropathy is very critical to successfully delay the superior stages of renal disease. Therefore, different related parameters have been used including BUN level, creatinine clearance, serum creatinine concentration and glomerular filtration rate (Levey *et al*., 1999; Lin *et al*., 2009; Paroni et al., 1990). Based on creatinine secretion, the quantity of plasma in blood revealed creatinine clearance rate is normally used for estimating GFR. Indeed, creatinine can be accurately calculated by relative measurement of substance in the blood and urine, subsequently GFR can be estimated by standard equations using the blood test results (Coresh and Stevens, 2006; Levey *et al*., 2006; Stevens *et al*., 2006). Normally, concentration of urea nitrogine (BUN), as one of liver waste from protein digestion, is approximately between 6-20 milligram for each 100 ml of blood. Unlike creatinine, filtered urea from kidney glomerular can be regulated, so the ratio between BUN and creatinine concentration in the blood reveals the efficiency of glomerular function and indicates the status of renal disorder practically in acute kidney injury (Feinfeld *et al*., 2002; Lyman, 1986). Recently, serum cystatin-c has been fund as a biomarker for kidney disease and early indicator for cardiovascular disease includes renal vessels (Bokenkamp and Herget-Rosenthal, 2004; Herget-Rosenthal *et al*., 2004; Krawczeski *et al*., 2010). In the current study, we further investigated levels of serum cystatin-c in patients with either renal or diabetic syndrome taken in consideration age and sexual variations. Renal and diabetic related factors were measured in more than eighty samples including levels of serum Cystatin-C and estimated GRF. Findings strongly reveal the sensitive connection between serum Cystatin-C and GFR in renal patients independent from diabetic syndrome. This observation indicates the beneficial role of serum Cystatin-C as an early indicator for diabetic nephropathy in renal patients and diabetic children.

## Materials and Methods

### Sample conditions

A total number of eighty-five patients with different renal and diabetic syndromes had been investigated for concentration of related factors including glucose, creatinine, BUN, cystatin-c and renal GFR. The blood samples were collected from eighteen patients contains eight males and ten females with only renal failure. Fifty-one samples were collected from

diabetic patients with normal renal function including thirty-five males and sixteen females. Other sixteen samples were collected for renal failure and diabetic patients including thirteen mails and three females. Additionally, ten samples were collected form normal peoples without any renal or diabetic syndromes to be used as a control.

**Laboratory investigation**

The laboratory investigations of blood samples were performed using Dimension Clinical Chemistry system (RXL Max. Siemens Healthcare Diagnostics),in Misr International Hospital All blood samples were collected in serum separators tubes (SST) and incubated for 30 min to be colt. Subsequently, samples were centrifuged and performed serums were collected and immediately frozen for the biochemical investigations. Serum creatinine was determined according to Jaffe reaction manufacture using wavelength of approximately, 520 nm (Paroni *et al*., 1990). Levels of glucose in blood samples were detected by using special tubes with fluoride, followed by centrifugation. Isolated plasma was incubated with hexokinase-glucose-6-phosphate dehydrogenase substrate, then relative concentration of glucose was measured at wavelength of approximately, 340 nm. Glycated hemoglobin was mentored by collecting blood samples on EDTA and using Turbidimetric inhibition immuno assay (TINIA) that uses turbidimetry as the measurement principle and used for many commercial immunoassays. Relative concentration of Cystatin-C was detected using quantitative sandwich immunoassay (Immunospec-USA Elisa kit) in which micro-titer plate with horseradish peroxidase (HRP)-conjugated polyclonal antibodies specific for Cystatin-C was prepared for stranded values. According to the normalization of standard values, Cystatin-C in blood samples were determined using the same micro-titer plate based on color change in spectrophotometrically at wavelength of approximately, 450 nm. Glomerular filtration rate (GFR) in blood samples was estimated dependent on MDRD formula for each sample.

**Staitistical analysis**

Microsoft Excel was used for statistical calculation, graphs, and histograms. GFR values was caculated according to MDRD (Modifecation of Diet of Renal Diseases) equation

eGFR (mL/min/1.73 $m^2$) = 186 X [(creatinine (mg/dL)]$^{-1,154}$ X (age)$^{-0,203}$X 0.742 (for women) (Levey et al., 1999).

**Results**

**Relative concentration of Cystatin-C and GFR values in elder patients**

To test the correlation between estimated GFR and secreted Cystatin-C in renal and diabetic elder patients, samples from renal or diabetic patients were collected and investigated for the relative concentration of glucose and glycated hemoglobin as an indicator for diabetic disorder. Concentrations of BUN, creatinine and Cystatin-C were also investigated as indicators for renal failure. Finally, GFR values were calculated according to Modification of Diet of Renal Diseases (MDRD) formula in each sample. Compared to

the normal peoples (non-renal and non-diabetic), glucose level in blood samples was in normal case confirmed the disappearance of diabetic disorder in tested renal, non-diabetic patients (Fig.1 A and B). Glucose level in diabetic patients was extremely high confirmed the diabetic syndromes in tested patients (Fig. 1 C). Likely, levels of creatinine and BUN were extremely high; however relative values of GFR were decreased in renal non-diabetic patients, approximately 6 folds down-regulation compared with normal and diabetic patients (Fig. 1 B). Interestingly, main concentration of Cystatin-C was increased in renal patient in compassion with normal and diabetic patients, approximately 6 folds up-regulation (Fig.1 A, B and C). This result indicates the contrary relationship between GFR and Cystatin-C in renal patients and demonstrates that Cystatin-C might be considered as an early indicator for renal failure independent from diabetic disorders.

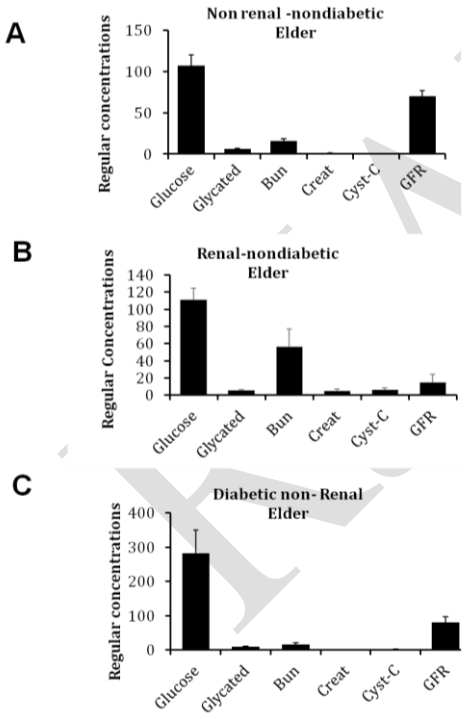


**Figure1. Relative concentrations of indicated factors in renal failure and diabetic elder patients. (A)** Regular concentrations of glucose, glycated hemoglobin, BUN, creatinine, cystatin-c and GFR values in control and normal patients. **(B)** The concentrations of indicated factors in renal non-diabetic patients. **(C)** The concentrations of indicated factors in diabetic non-renal patients. Error bars indicates the standard deviation (SD) of individual samples in each group. Data are representative of two independent experiments.

**Investigation of Cystatin-Cand GFR in adult patients**

To further valdate our observation, Cystatin-C and GFR values were also investgated in adult patients with renal or diabitic syndrom. Samples were collected and prepared for detection of related factors. The result reavels that, levels of glucose and renal factors were extremely increased in diabetic and renal failure patients, respectively (Fig. 2 A and B). Typically, GFR values were strongly decreased in renal failure patients in comparison with diabetic patients without renal syndrome, more than10 folds down-regulation.Unlik eGFR, Cystatin-C concentrations were quite increased in renal failure patients compared to diabetic patient, approximately, 3 folds up-regulation, indicating the potential correlation with GFR (Fig. 2 A and B). The result from adult patients further confirms the conflicting relationship between Cystatin-C and GFR in renal failure patient in dependent from diabetic syndromes.

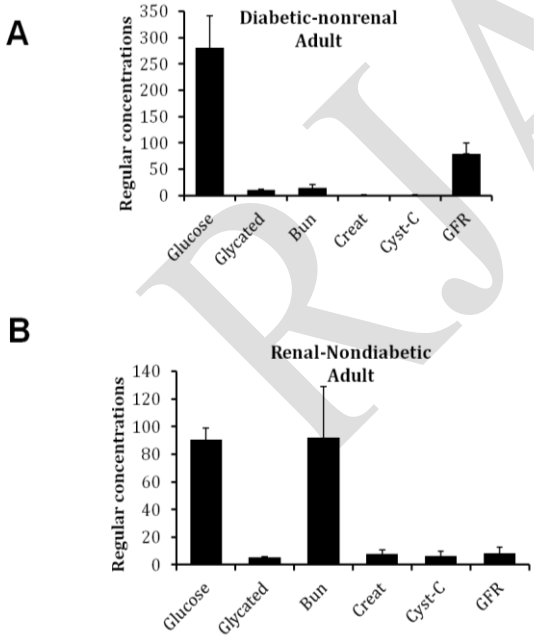


**Figure 2. Relative concentrations of indicated factors in renal and diabetic adult patients.** (A) Regular concentrations of glucose, glycated hemoglobin, BUN, creatinine, cystatin-c and GFR values in diabetic non-renal patients. (B) Concentrations of indicated factors in renal non-diabetic patients. Error bars indicates the (SD) of individual samples in each group. Data are representative of two independent experiments.

**Linkage between Cystatin-C and GFR in children patients**

In children patients with renal failure or diabetic disorder, levels of Cystatin-C and GFR values were investigated in addition to the other related factors. As usual, level of glucose was increased in diabetic children, while revealed normal level in renal non-diabetic children. All renal related factors including creatinine and BUN were increased in renal failure children (Fig. 3 A and B). GFR values were quite reduced in renal children in comparison with non-renal children, approximately 4 folds down-regulation. Interestingly, concentration of Cystatin-C was extremely increased in renal, non-diabetic children, more that 10 folds up-regulation (Fig. 3 A and B). These results exhibit the negative correlation between GFR and Cystatin-C in renal children and suggest that Cystatin-C is a crucial factor considered in renal diagnostic in children. Taken together, these findings indicate the essential role of Cystatin-C in renal failure diagnostic and further confirm the negative correlation between cystatin-c and renal GFR. Additionally, these data strongly suggest the possible consideration of Cystatin-C as one of the early indicator factors reflecting renal disfunction independent from diabetic disorder.

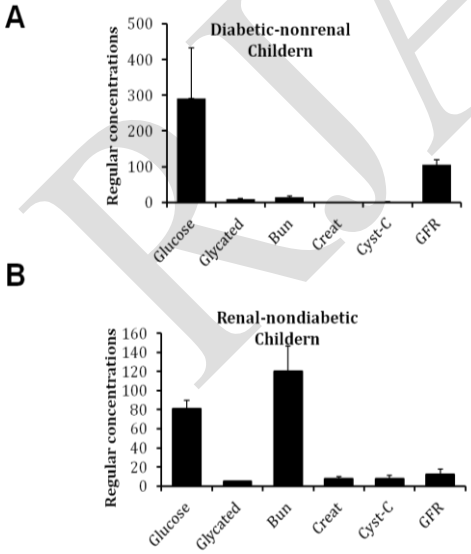


**Figure 3. Relative concentrations of indicated factors in renal and diabetic children patients.** (A) Regular concentrations of glucose, glycated hemoglobin, BUN, creatinine, cystatin-c and GFR values in diabetic non-renal children. (B) Concentrations of indicated factors in renal non-diabetic children. Error bars indicates the (SD) of individual samples in each group. Data are representative of two independent experiments.

**Discussion**

The current data further confirm the correlation between glomerular filtration rate and levels of serum Cystatin-C as an early indicator for renal failure independent from glucose level in diabetic patients. Generally, renal disease detection is based on GFR values which typically estimated by MDRD formula. Unlike serum Cystatin-C, a level of serum creatinine is interrupted in sexual and age dependent manner indicating the lacking of using only serum creatinine concentration in early detection of renal disease (Finney *et al*., 2000; Perrone et al., 1992). Cystatin-C, also known as cystatin 3, is a low molecular weight neuroendocrine protein encoded by *CST3* gene (Watanabe et al., 2006). Over-expression of Cystatin-C refers to various human disorders and diseases such as cardiovascular fibroblasts, brain disorder and male reproductive disease (Babiloni *et al*., 2006; Jiborn *et al*., 2004; Xie et al., 2010). Depending on the efficiency of kidney vessels, filtration rate of CystatinC from blood can be regulated. Interruption of filtration rate resulted in high levels of Cystatin-C in blood might expose the initiation of renal syndrome or glomerular nephropathy (Bokenkamp and Herget-Rosenthal, 2004; Krawczeski et al., 2010; Madero *et al*., 2006). Therefore, the correlation between GFR and levels of Cystatin-C must be considered in diagnostic progress of renal disease and detection of early glomerular nephropathy.

In the current study, blood samples were collected from children, adult and elder patients with renal and/or diabetic syndromes in order to deeply investigate the possible involvement of serum Cystatin-C on the early recognition of renal failure. Further, GFR was calculated depending on MDRD formula according to equation indicated by Levey et al., 1999. Our findings showed approximately 10 times less of GFR values in renal failure patients in comparison with normal and diabetic patients. In contradiction to GFR, levels of serum Cystatin-C showed approximately 5 times more in renal failure patents. Like Cystatin-C, serum creatinine showed increasing levels in renal syndrome patients compared to normal and diabetic patients. Importantly, levels of serum creatinine in renal failure children was about 2 times less than level of serum Cystatin-C indicating the effectiveness of age variation on serum creatinine concentration even in renal failure patients. This observation strongly indicates that serum cystatin-c is more constant candidate than serum creatinine on the early recognition of renal disease practically in children. Renal disease in children is a serious complication connected with long-term diabetes. Over all 15 years of uncontrolled diabetes mellitus in children, renal failure is initiated and diabetic nephropathy is performed in more than 30% of diabetic patients. Therefore, regular screening and controlling of diabetes might improve the ability of renal function (Chiarelli and Mohn, 2002; Fekete and Vannay, 2014; Tang *et al*., 2013). Importantly, early detection of renal syndromes in diabetic children is extremely helpful in diagnostic progress and identification of efficacious treatment that might prevent or delay renal failure syndromes. Here, results exposed from diabetic and renal children strongly indicate that

serum Cystatin-C is a critical factor that can reveal the efficiency of renal function. Additionally, level of serum Cystatin-C was dramatically increased up to 10 folds in renal children compared with normal or diabetic children indicating the connection between level of serum cystatin-c and efficiency of renal filtration rate. Taken together, these findings further confirm the correlation between glomerular filtration rate and serum Cystatin-C into various ages and indicating the beneficial role of serum Cystatin-C in early detection of renal syndromes particularly in diabetic children.

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