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Serum Cystatin C in Type 2 Diabetic Patients with Early Renal Damage

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Diabetic nephropathy is one of the most common complications in diabetes mellitus (DM). The ability to assess renal function in diabetes patients rapidly and early is of major importance. Nowadays, cystatin C (cys C) is introduced as a new marker for diagnosis of early renal damage. The purpose of this study was to study serum cys C in type 2 diabetic patients with early renal damage. This is a hospital-based, cross-sectional analytical study involving 50 cases of type 2 diabetic patients attending the Diabetic Clinic of Mandalay General Hospital. In this study, most cases were females with male to female ratio of 1:2. Mean age was 59±11.9. Mean values of serum cys C, albumim creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) were 0.89±0.37 mg/l, 18.46±16.47 mg/g and 92.34±22.63 ml/min, respectively. In this study, 60% of cases were eGFR 60-90 ml/min and 40% of cases were eGFR >90 ml/min. And then, 82% of cases were normoalbuminuria and 18% of cases were microalbuminuria. Serum cys C was negatively correlated with eGFR (r=-0.0235, p=0.1) and positively correlated with urine for ACR (r=0.177, p=0.219). In addition, serum cys C was positively correlated with normoalbuminuria (r=0.188, p=0.238) and negatively correlated with microalbuminuria (r=-0.008, p=0.984). But these are not statistically significant. Therefore, this study is concluded that serum cys C was higher in both normoalbuminuric and microalbuminuric type 2 diabetic patients. The correlations of serum cys C with microalbuminuria and normoalbuminuria were not statistically significant. Therefore, it is controversial to say that serum cys C can be used as early detection marker of renal damage in type 2 diabetic patients in this study.

Keywords: Cystatin C, eGFR, Albumim creatinine ratio, Renal damage, Type 2 diabetes mellitus

INTRODUCTION

The incidence of diabetes mellitus increases in most countries of the world. The global prevalence of diabetes among adults over 18 years of age has risen from 4.7 percent in 1980 to 8.5 percent in 2014 (WHO, 2016). WHO projects that diabetes will be the 7th leading cause of death in 2030.[1] Increasing trend of prevalence of diabetes was reported in Myanmar from 1980 to 2014. It indicates 5.4 percent to 8.5 percent in men and 6.9 percent to 8.2 percent in women, respectively.[2] In Mandalay General Hospital (MGH), there were 1189, 1323 and 1704 cases of diabetes mellitus in 2013, 2014 and 2015, respectively.[3] One of the most common long-term complications of diabetes is kidney damage. Diabetic nephropathy increases cardiovascular disease risk by 20-40 folds. In the past two decades, the prevalence of the end-stage renal failure has increased dramatically.[4] Therefore, it is necessary to detect the early renal damage as early as possible. Estimated glomerular filtration rate (eGFR) is used to screen and detect for early renal damage. It can be easily calculated by Modification of Diet in Renal Disease (MDRD) equation.[5] Two positive tests for albumin in the urine over several weeks indicate persistent albuminuria, a first sign of diabetic kidney disease.[6] Microalbuminuria can be a forerunner of diabetic nephropathy.

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It is believed that urine for microalbumin excretion may be affected by position, exercise, urinary tract infection, stress response and protein intake.[4] Urinary albumin creatinine ratio (ACR) is the most appropriate first-line test for the detection of proteinuria in diabetic nephropathy.[7]

Cystatin C has been proposed as an alternative endogenous glomerular filtration marker. It is cationic nonglycosylated cysteine proteinase inhibitor and is produced at a constant rate by all nucleated cells and owing to its low molecular weight, is freely filtered by the glomerulus and almost completely reabsorbed and degraded by proximal tubular cell.[7] Serum cys C production is not affected by age, sex and muscle mass.[8]

In a study performed in China on 51 type 2 diabetic patients, cys C is superior to serum creatinine and creatinine clearance for detecting impaired GFR.[9] In India, cys C was assessed as a marker of early diabetic nephropathy in Indian subjects with glucose intolerance. In type 2 diabetic subjects, cys C and eGFR appear to be useful markers of early renal damage.[10]

In a study done in Iran, serum cys C was higher in diabetic patients with GFR <60 ml/min than other healthy subjects. A borderline significant correlation between cys C and eGFR ($p=0.05$) but highly significant with microalbuminuria ($p=0.014$) was observed. However, there was not any correlation between cys C and HbA1c. Therefore, measurement of serum cys C concentration is a useful marker for screening of diabetic nephropathy in diabetic patients, but it cannot be used for monitoring of these patients.[4]

In Myanmar, the incidence of diabetes mellitus is increasing because the population is growing and adopting less healthy lifestyle. Diabetic nephropathy is one of the most common diseases and is the major cause of worldwide diabetes-related mortality. The ability to assess renal function in diabetic patients rapidly and early is of major importance. Many biochemical parameters are used to detect complications and prognosis of diabetes mellitus. Among them, cys C is early sensitive marker for kidney disease. It can be easily detected in human serum and can detect renal damage earlier than the serum creatinine.

This study was aimed to determine serum cys C in type 2 diabetic patients attending the Diabetic Clinic of Mandalay General Hospital. In Myanmar, there was no previous study of serum cys C in type 2 DM patients. The findings from this study could be of help for clinicians to predict the early detection of renal damage in type 2 diabetic patients.

MATERIALS AND METHODS

It is the hospital-based, cross-sectional analytic study. Type 2 diabetic patients with early nephropathy attending the Diabetic Clinic of MGH from July 1, 2016 to June 30, 2017 were included in this study except patients with exclusion criteria. Sample size was calculated by using Statistic Software PASS (Power Analysis and Sample Size) 13. Total 50 cases of type 2 diabetic patients with early renal damage were recruited by consecutive sampling at Diabetic Clinic of MGH.

Sample collection and preparation

Type 2 diabetic patients were selected according to the inclusion criteria (patients with type 2 diabetes mellitus with early renal damage) and exclusion criteria (over the age of 70 years, under the age of 18 years, currently taking some drugs such as gentamycin, cisplatin, cefoxitin, cimetidine, trimethroprim, steroid, insulin, history of some disease such as urinary tract infection, liver disease, thyroid disorder, malignancy). After getting informed consent, calculation of eGFR was done by MDRD equation by using previous creatinine result of the patient. From the patients with eGFR ≥60 ml/min, random urine samples about 10 ml was collected in screw-capped sterile bottles without any preservatives. Macroalbuminuria was excluded by urine dipstick test. History taking according to pro-forma was done. A total of 4 ml of venous blood sample were collected into the plain tube under aseptic condition for serum cys C. Serum was separated by centrifuging and stored exactly at -20°C until analysis.

Serum cys C and microalbuminuria were examined by fluorescence immunoassay analyzer (ichroma™). Urine for creatinine was examined by fully chemical auto-analyzer (ABX Pentra-400). Urine for ACR was calculated. And then, data analysis of urine for albumin creatinine ratio and serum cys C was done.

Operational definitions

Early renal damage

Chronic kidney damage (CKD) stages 1 and 2 regard as early kidney disease. Stage 1 CKD patients have essentially a normal or elevated GFR (90+ ml/min) and normo- or micro-albuminuria, while stage 2 patients have a mildly reduced GFR (60-89 ml/min) and microalbuminuria.[11]

eGFR

Estimated glomerular filtration rate was calculated by original MDRD equation;

$$eGFR = 175 \times (S_{Cr})^{-1.154} \times (age)^{-0.203} \times 0.742 \text{ [if female]} \times 1.210 \text{ [if Black]}.$$[12]

Urine for albumin creatinine ratio (ACR)

It is ratio of urinary albumin to urinary creatinine. There are three cut-off values of ACR for urine.[13]

ACR <30(mg/g) - Normoalbuminuria
ACR 30 - 300(mg/g) - Microalbuminuria
ACR >300(mg/g) - Macroalbuminuria

Reference range of cystatin C[14]

18-50 years - 0.56-0.90 mg/l
51-70 years - 0.58-1.09 mg/l

Data processing and analysis

Data entry was done by using spread sheet of Microsoft Office Excel 2010. Data was analyzed by Stata 13. Correlation was analyzed using Pearson correlation test between serum cystatin C and urine for ACR.

Ethical consideration

Ethical approval was obtained from Ethic Review Board of University of Medicine, Mandalay.

RESULTS

Total 50 cases of type 2 diabetic patients who fulfilled the inclusion criteria were studied. There were17 males (34%) and 33 females (66%). Mean age was 59±11.9 years. Mean values of serum cys C, ACR and eGFR were 0.89±0.37 mg/l, 18.46±16.47 mg/g and 92.34±22.63 ml/min, respectively. Thirty (60%) cases were eGFR 60-90 ml/min and 20 (40%) were eGFR >90 ml/min, respectively and 9(18%) were microalbuminuria and 41(82%) were normoalbuminuria (Table 1).

Table 1. Frequency distribution of demographic data and clinical parameters of type 2 diabetic patients

Variables	Frequency (n=50) (%)
Sex	
Male	17(34)
Female	33(66)
Age (yr)	
20-39	7(14)
40-59	25(50)
≥60	18(36)
eGFR (ml/min)	
60-90	30(60)
>90	20(40)
Urine for ACR	
microalbuminuria	9(18)
normoalbuminuria	41(82)

eGFR=Estimated glomerular filtration rate
ACR=Albumin creatinine ratio

Figure 1 shows that levels of serum cys C distribution of type 2 diabetic patients in different eGFR. In eGFR 60-90 ml/min group, there were 30 type 2 diabetic patients and in eGFR >90 ml/min group, there were 20 type 2 diabetic patients.

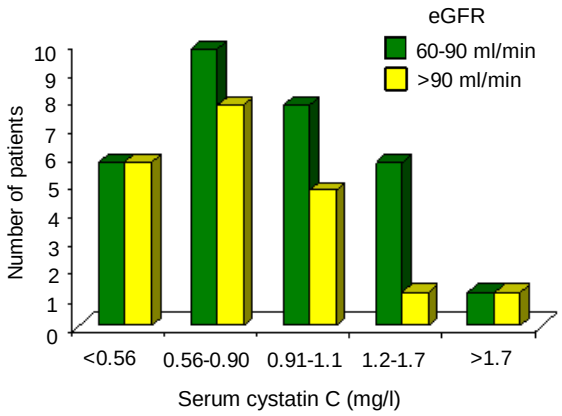

Fig. 1. Levels of serum cystatin C distribution of type 2 diabetic patients in different estimated glomerular filtration rate (eGFR)

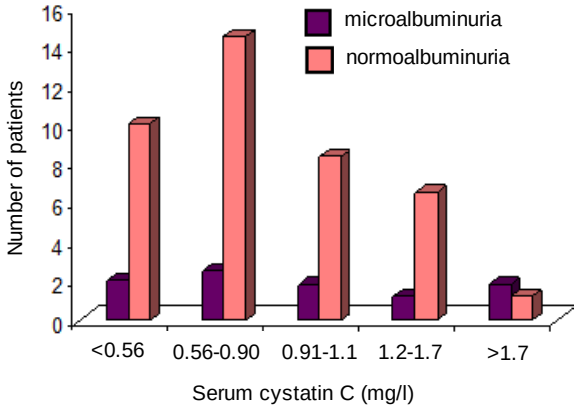

Fig. 2. Levels of serum cystatin C distribution of type 2 diabetic patients in different albuminuria

Figure 2 shows that levels of serum cys C distribution of type 2 diabetic patients in different albuminuria. In this study, there were 9 microalbuminuric patients and 41 were normoalbuminuric patients.

The Pearson correlation coefficient (r) of serum cys C with eGFR was -0.0235, 95% confidence interval (CI) was -0.009-0.001 and p value was 0.1. The Pearson correlation coefficient (r) between serum cystatin C and urine for ACR was 0.177, 95% confidence interval (CI) was -0.002-0.011 and p value was 0.219.

The correlation (r) between serum cys C and normoalbuminuria was 0.188, 95% confidence interval (CI) was -2.525-9.853 and p value was 0.238. The correlation (r) between serum cys C and microalbuminuria was -0.008, 95% confidence interval (CI) was -22.084-21.701 and p value was 0.984.

DISCUSSION

Diabetes mellitus (DM) affects more than 120 million people worldwide, and it is estimated that it will affect 370 million by the year 2030.[15] Chronic kidney disease is increasing worldwide, with higher prevalence in developing countries; diabetic nephropathy (DN) is a common underlying cause.[16]

The GFR is considered the most accurate measurement of kidney disease and is reduced before the onset of clinical symptoms.[17] Microalbumin-uria has attracted much clinical attention in recent years due to its prediction of progression to diabetic nephropathy.[18] The National Kidney Foundation recommends the use of spot urine ACR obtained under standardized conditions to detect microalbuminuria. The ACR is a more convenient test for patients.[19] Serum cys C is considered as a good marker of GFR as it is not influenced by gender, muscle mass, age and protein intake; cys C is preferable to creatinine.[20]

In the present study, the number of women were more than men. This may be due to smaller sample size and the combined effect of a greater number of elderly women than men in general population. This study found that younger patients with renal damage can also have high serum cys C levels and elderly patient without renal damage can also have low serum cys C levels. The two persons who were in highest serum cys C level (>1.7 mg/l) were at the age of 38 years and 47 years.

Mean value of urine for ACR was 18.59 ±16.71 mg/g. It is in the marginal value of normoalbuminuria and microalbuminuria. Mean eGFR in this study was 92.34± 22.63 ml/min. Most of the patients in this study were normoalbuminuric patients. Total 15(30%) subjects had elevated serum cys C levels in this study.

The present study showed mean serum cys C value in type 2 DM with normoalbuminuria was 0.87±0.33 mg/l and that with microalbuminuria was 1.01±0.55 mg/l. So, according to this finding, cys C was higher in microalbuminuria than normoalbumiuria.

Figure 1 shows that both groups of eGFR patients can undergo high serum cys C level. Figure 2 shows the higher serum cys C levels in both normoalbuminuric and microalbuminuric patients. According to these data, increased serum cys C level can be found in early renal damage of type 2 diabetes patients. In a study done on 78 cases of type 2 DM patients, the values of cys C were significantly increased in the normoalbuminuria group.[21] Therefore, these data are compatible with those of the present study.

In the present study, cys C showed negative correlation with eGFR (r=-0.0235, p=0.1) and positive correlation with urine for ACR (r=0.177, p=0.219). But these correlations were not statistically significant. A study in Egypt done on type 2 DM patients also found that there was a significantly negative correlation between cys C and glomerular filtration rate (r=-0.65, p=0.001) and positive correlation between cys C and urine for ACR (r=0.78, p=<0.001).[22] Negative correlation with eGFR shows that serum cys C is an early marker of nephropathy.[23] Therefore, a statistically insignificance of negative correlation in present study may be due to small sample size or the study population with eGRF ≥60 ml/min.

In this study, serum cys C was positively correlated with normoalbuminuria (r=0.188, p=0.238) and negatively correlated with microalbuminuria (r=-0.008, p=0.984). But

these are not statistically significant. The negative correlation of serum cys C with microalbu-miuria may be due to small sample size. There were only 9 microalbuminurictype 2 DM patients in the present study. The positive correlation between serum cys C and normoalbuminuria was described as serum cys C can be used as diagnostic marker in early renal damage of type 2 DM patients but it was not statistically significant in the present study. A study done in Iran on 126 cases of type 2 DM patients observed that there was a positive correlation between serum cys C and microalbuminuria (r_S=0.22, p=0.014).[4]

The limitations in this study are small sample size, limited sample population and study area. Therefore, it is recommended that a large sample size with extended study population and area should be used to overcome these limitations and prospective studies are needed to use the serum cys C as early diagnostic marker in renal damage.

Therefore, it is controversial to say that serum cys C can be used as early detection maker of renal damage in type 2 diabetic patients according to this study. This study, even with many limitations might be helpful and useful, to some extent, in the detection of early renal damage by simple and convenient laboratory detection of serum cys C.

Competing interests

The authors declare that they have no competing interests.

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