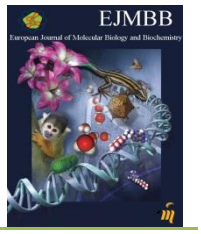
e - ISSN - 2348-2206
Print ISSN - 2348-2192

European Journal of Molecular Biology and Biochemistry

Journal homepage: www.mcmed.us/journal/ejmbb



COMPARISON OF SERUM CREATININE AND SERUM CYSTATIN C IN DIABETIC NEPHROPATHY

Gaffar Sarwar Zaman

Assistant Professor, Department of Clinical Biochemistry, Government College of Medicine, King Khalid University, Abha, Kingdom of Saudi Arabia.

Article Info

Received 23/07/2014
Revised 16/08/2014
Accepted 19/08/2014

Key words:- Serum Creatinine, cystatin C, nephropathy, diabetes mellitus.

ABSTRACT

Serum Creatinine estimation is dependent upon various factors and hence not an accurate marker of renal function. Recently Serum Cystatin C estimation is found to be comparable with Serum Creatinine estimation as a marker of renal function. But its use in diabetic nephropathy is still inconclusive. Two groups, one with long-standing non-insulin-dependent diabetes mellitus (type II), and one control, each containing 40 cases, were considered for the study. A significant increase of all the measured parameters in comparison to the control group, was observed. A more significant increase ($p<0.001$) was observed in cystatin C than in Serum creatinine ($p<0.05$). The present study demonstrated that determination of serum cystatin C might be useful in the detection of incipient diabetic nephropathy and is a potentially better marker than creatinine during advanced diabetes mellitus type 2.

INTRODUCTION

Cystatin C (Cys-C) is an endogenous 13-kDa protein filtered by glomeruli and reabsorbed and catabolized by tubular epithelial cells with only small amounts excreted in urine and reported to be generated at a relatively constant rate irrespective of muscle mass. It is encoded by the 'housekeeping type" CST3 gene (belonging to the type 2 cystatin gene family), and produced by all nucleated cells at a constant rate [1,2]. It is freely filtered by the glomerulus and is largely reabsorbed and catabolised in the proximal tubules. The low molecular mass of Cys-C, in combination with its stable production rate, indicates that the plasma concentration of Cys-C is almost exclusively determined by the glomerular filtration rate (GFR), what makes cystatin C an excellent indicator of GFR9 [3-5]. Most studies have shown that serum cystatin C levels more closely correlated with GFR than serum creatinine; however, the few studies that compared serum Cys-C with estimates based on serum creatinine, age, sex, and race showed them to be comparable [6-9]. Several factors affect serum creatinine level other than GFR, including its generation from muscle metabolism. GFR-estimating equations, such as the Modification of Diet in Renal Disease (MDRD) Study equation, include age, sex, and race to account for average differences in muscle mass in subgroups. These other causes of creatinine generation lead to imprecision in the estimates [10,11]. Plasma or Cys-C has been proposed as a marker for GFR [12-15], and several commercially available automated procedures for rapid determination of Cys-C have been reported [16-19]. Later, many other studies showed that serum cystatin C is a better estimator of GFR than serum creatinine in different populations [20-24]. As mentioned before, most of the studies focused on patients with mild to moderate renal dysfunction, and the various developed cystatin C based equations have still questionable accuracy compared mainly to radionuclide estimation of GFR [25]. Andersen et al. reviewed clinical studies in children evaluating serum Cys-C, Cys-C based formulas, and plasma creatinine or creatinine based formulas against an exogenous reference

Corresponding Author

Gaffar Sarwar Zaman
Email: - drgszaman@gmail.com

method and concluded that Cys-C based formulas are comparable with creatinine based formulas [26].

Cys-C is currently being investigated for the prediction of AKI in patients with cardiac surgery [27], advanced liver diseases [28], and patients undergoing liver transplantation [29]. After age 1, serum Cys-C concentration is constant, but higher values are found in the newborn period. In full-term newborns, Cys-C progressively declines over the first week of life, and less significantly, over the first month [30]. Cys-C–GFRhas been reported to be more accurate in children with cancer [31] and in patients with spina bifida or spinal injury [32,33]. It is said that serum cystatin C concentration varies in pregnancy, because it is not consistently produced. In preeclampsia, however, altered kidney function is more likely to be detected by Cys-C–GFR than by creatinine-based formulas [34]. GFR declines with age and cystatin C may better reflect true kidney function in older people because muscle mass does not influence it [35]. After age 50, reference values of serum cystatin C concentration are higher. The prevalence of stage 3 CKD in an elderly population when GFRis estimated by the MDRD Study equation, is significantly higher than the prevalence obtained when Cys-C– GFRequations are used [36].The purposes of this study were to investigate whether serum Cys-C levels are increased in diabetic nephropathy patients, and to further determine whether Cys-C can be used as an early biomarker for predicting diabetic nephropathy in advanced diabetic cases.

MATERIALS & METHODS

The group with non-insulin-dependent diabetes mellitus (type II) included 40 cases. A control group of 40 healthy adults without inflammatory states nor abnormalities in lipids and carbohydrate metabolism, in routine medical check-ups, were also examined.

Body mass index (BMI) was calculated for each. All patients needed to have stable renal function (by measuring daily serum creatinine) over three successive days.

The diagnosis of DM was based on WHO criteria, i.e. a fasting plasma glucose level > 7.0 mmol/L or > 126 mg/dL, or a 2-h postprandial plasma glucose level > 11.1 mmol/L or > 200 mg/dL on more than one occasion, with symptoms of diabetes. For definition of the glycemic condition of the patients, fasting blood glucose in plasma (short-term control) and concentration of glycated hemoglobin (GHb) in hemolysates (long-term control) were determined. Fasting serum was obtained to measure creatinine and Cys-C. Serum creatinine was measured on the same day by the Jaffe colorimetric method. An aliquot of serum was kept in refrigerator at -4ºC until Cys-C was measured. Serum Cys-C was measured by particle-enhanced turbidometric immuno-assay (PETIA) using PET kit. The GFR was calculated using ser Cys-C and creatinine separately and the values were compared with the measured GFR by 99mTC-DTPA clearance. Patients were divided into 3 groups according to albumin excretion rate)5: with normoalbuminuria (albumin excretion below 30 mg/24 h), with microalbuminuria (albumin excretion between 30 and 300 mg/24 h) and the group with macroalbuminuria (albumin excretion over 300 mg/24 h). The data were expressed as mean ± standard deviation (range). Correlations between quantitative data were determined using Pearson's test. *P* values less than 0.05 were considered statistically significant. For statistical analysis, the SPSS R 9.0 (Statistics Package for social science) program was used.

RESULTS

Table 1. Anthropometric measurements of the controls and advanced diabetes mellitus type 2 cases

Parameters	Controls	Cases
Age (in years)	56.7 ± 14.68	54 ± 18.38
BMI (kg/m^2)	25.6 ± 3.1	26.3 ± 2.7
WC (cm)	94.2 ± 6.5	93.4 ± 7.9
WHR	0.94 ± 0.09	0.95 ± 0.04

In the present study, there were 40 advanced diabetes mellitus type 2 cases and 40 controls. Out of 80 patients, the controls age group was 56.7 ± 14.68 years and the cases age group was 54 ± 18.38. The BMI of the controls had an average of 25.6 ± 3.1 kg/m^2 and the BMI of the cases was 26.3 ± 2.7 kg/m^2. The waist circumference was 94.2 ± 6.5 cm. in the controls and 93.4 ± 7.9 cm in the cases and the WHR was 0.94 ± 0.09 in the controls and 0.95 ± 0.04 in the cases.

Table 2. Primary diagnosis according to the associated conditions

Parameters	Controls	Cases
Plasma glucose (mg/dL)	84±18	143±29
Glycated hemoglobin (%)	5.7	9.8
Creatinine clearance (ml/min)	99.73	68.46

Table 3. Comparison of accuracy of Glomerular filtration rate, Serum Creatinine and Serum Cystatin C in controls and cases.

Parameters	Controls	Cases	p-value
Glomerular filtration rate (GFR) (ml/min/1,73 m2)	114± 5.60	81.35±18.43	p<0.05
Cystatin C (mg/l)	0.88 ± 0.24	5.1± 1.58	p<0.001
Creatinine (mg/dl)	1.11 ± 0.78	4.0 ± 2.24	p<0.05

Serum cystatin C and serum creatinine (mg/dL) were detected significantly higher in cases ($p< 0.005$, $p<0.001$), when compared to the other group. Also, GFR (mL/min) was detected significantly lower in cases ($p<0.005$), when compared to the control group. A more significant increase (p<0.001) was observed in cystatin C than in serum creatinine (p<0.05).

ACKNOWLEDGEMENT

I would like to express my deep gratitude to Dr. Fawzia Zaman (MBBS, MD) for her patient guidance, enthusiastic encouragement and useful critiques of this research work. I would also like to thank her for providing the data and helping me in the analysis of the data. I would also like to extend my thanks to the technicians of her laboratory for their help in offering me the resources in completing the research work.

DISCUSSIONS

Some studies have suggested that Cys-C may be superior to serum creatinine or creatinine based estimating equations as a marker of kidney function, whereas others have found no difference [36- 39]. The results of this study clearly indicate that Cys-C levels were significantly increased in all diabetic nephropathy patients, and serum Cys-C is a more accurate and efficient marker for detecting renal involvement than serum creatinine.

Its low molecular weight (13.3 kDa) and positive charge at physiological pH levels facilitate its glomerular filtration. Subsequently, it is reabsorbed and almost completely catabolized in the proximal renal tubule [40,41]. Therefore, because of its constant rate of production, its serum concentration is determined by glomerular filtration [42,43]. Moreover, its concentration is not influenced by infections, liver diseases, or inflammatory diseases. The use of serum Cys-C as a marker of GFR is well documented, and some authors have suggested that it may be more accurate than serum creatinine for this purpose [44-50]. The results of Cys-C and SCr in the present study were consistent to those of other research on hypertension, diabetes and kidney transplantation. Other Limitations of Creatinine are - Acute changes in kidney function are not immediately apparent. Creatinine excretion is due not only to filtration (90%–95%) by the kidney but also to secretion (5%–10%) by the distal tubule. If the patient with advanced CKD takes a substance that blocks distal tubule secretion of creatinine (eg, trimethoprim, cimetidine, cefoxitin), the serum creatinine level will increase abruptly, but the actual GFR will not change [51]. Extra-renal elimination of creatinine occurs. The plasma Cys-C concentration of our control group and cases was similar to that obtained by Erlandsen et al [52] for the serum of blood donors and Agnieszka Piwowar et al [53] for plasma Cys-C. We found a weaker association of age with cystatin C level than with serum creatinine level, consistent with this a priori hypothesis, as well as with most published cystatin C–based estimating equations that do not include terms for age, sex, or race [54-58]. High Cys-C concentrations predict substantial increased risks of all-cause mortality, cardiovascular events, and incident heart failure among ambulatory persons with CHD [59,60].

Serum Cys-C level was shown to have a stronger association with mortality and cardiovascular disease than serum creatinine level, particularly in studies of older adults [61-64]. Another contributing factor may be that serum Cys-C levels are subject to variations when measured by different methods (i.e. Gentian method DAKO method) [65]. Additionally, there is an ongoing controversy regarding the biological variation of Cys–C levels. It has been suggested that Cys-C serum concentrations may exhibit a high within-subject variation [66,67].

CONCLUSION

Our results suggest that the determination of the serum concentration of cystatin C may be useful in the detection of incipient nephropathy in patients with non-insulin-dependent diabetes mellitus and is a potentially better marker than creatinine. In conclusion, this analysis has shown that cystatin C based estimates of GFR are more reliable than the traditional creatinine based estimates.

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