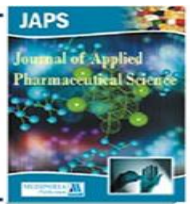
Journal of Applied Pharmaceutical Science
Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 10-09-2011
Revised on: 15-09-2011
Accepted on: 22-09-2011

Is Cystatin C a powerful Predictor of Cardiovascular Diseases in patients with type 2 Diabetes Mellitus? (Study on Egyptian patients)

Hesham Rafat, Sameh H. Soror, Zeinab Hassan and Ashref Ismaail

Hesham Rafat,
Sameh H. Soror, Zeinab Hassan
Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, University of Helwan Ain Helwan, University Campus Post code 11795, Cairo, Egypt.

Ashref Ismaail
National institute of diabetes and endocrinology, El Kaser Elainy Cairo, Egypt.

For Correspondence:
Sameh H. Soror
Department of Biochemistry and molecular biology, Faculty of Pharmacy, University of Helwan, Ain Helwan, University Campus, Post code 11795, Cairo, Egypt.

ABSTRACT

Cystatin C is a non-glycosilated protein. It is used mainly as a biomarker of renal functions. Diabetes mellitus has been associated with serious complications. Diabetic nephropathy is the main risk factor for morbidity in diabetes mellitus. This study was conducted to assess the clinical value of Cystatin C (CysC) in the diagnosis of cardiovascular complications and diabetic nephropathy in type 2 Egyptian diabetic patients. Serum CysC level was determined in three groups of patients; Diabetics, diabetic nephropathy and diabetic cardiovascular, using ELIZA technique. CysC showed higher sensitivity in early kidney dysfunction and the different stages of nephropathy relative to the control group. C-reactive protein (CRP) showed significant difference in diabetic hypertensives group and in diabetic hypertensives suffering cardiac problems when compared to the control. We found that CysC had higher sensitivity, specificity and area under the curve than creatinine, microalbuminuria and CRP CysC is one of the most efficient markers for diagnosis of diabetic complications and may be used to assess mild kidney impairment in healthy individuals with high muscle mass.

Key words: Diabetic complications, Cystatin C, microalbuminuria, ACR, CRP, and GFR_{Cys}.

INTRODUCTION

Cystatin C (CysC) belongs to the cystatin superfamily of cysteine protease inhibitors. It is unique among all known cystatins that is produced at a constant rate by nucleated cells (Shlipak et al., 2006). In contrast to serum creatinine, which is influenced by different factors such as age, sex, muscle mass and inflammatory processes, serum CysC level is less affected by these factors (Cholongitas *et al*., 2007; Finney *et al*., 2000; Vinge *et al*., 1999). The low molecular weight (13KDa) and the positive charge of CysC at physiological pH help its glomerular filtration (Visvardis *et al*., 2004). After filtration CysC is reabsorbed and catabolized by the tubular epithelial cells. Hence, urinary clearance of CysC cannot be measured (Stevens *et al*., 2006). Serum CysC is superior to serum creatinine as a marker of renal function (Fujisawa *et al*., 2010). Diabetic nephropathy is the major risk factor for morbidity and mortality in diabetes mellitus. It is recommended that serum creatinine should not be used as diagnostic marker for kidney function alone but rather used to estimate the glomerular filtration rate (GFR) and stage the level of chronic kidney disease (CKD) (American Diabetes Association 2009). Atherosclerosis is an inflammatory disease of the cardiovascular system, which is characterized by extensive remodeling of the extracellular matrix of arterial walls. It has been implicated that an imbalance between the

expression of cathepsines and their endogenous inhibitor CysC is one of the most important mechanisms in atherogenesis. Several recent studies suggest that chronic kidney disease is an independent risk factor for ischemic heart disease, particularly among participants with established cardiovascular disease or those who are at increased cardiovascular risk. (Eriksson et al., 2004**).**
This study will evaluate the use of CysC as biomarker for early diagnosis of cardiovascular complications and diabetic nephropathy in type 2 diabetic patients.

MATERIALS AND METHODS

The study was carried out on 81 (type 2) diabetic patients. They were selected from the outpatients' clinic of the National Institute of Diabetes and Endocrinology, El Kasr eleiny, Cairo, Egypt. Studied subjects were classified into three different groups; control group of 18 diabetic patients, diabetic nephropathy group composed of 23 patients and 40 patients in the diabetic cardiovascular group.

Diabetic nephropathy group was selected by gross albuminuria < 300 mg/dl with no evidence of dialysis or kidney transplantation. Subjects in this group have normal blood pressure and showed no symptoms of any cardiac disease. Diabetic nephropathy group was subdivided into diabetic nephropathy stage 1 -CKD1- (16 patients) and diabetic nephropathy stage 2 -CKD2- (7 patients) based on their GFR; normal GFR with microalbuminuria and GFR of 60-89 ml/min with microalbuminuria respectively.

Diabetic cardiovascular group was selected according to their hypertension history (BP>140/90), ECG changes, peripheral vascular disease (ischemia, ulcer, cerebral strokes, intermittent claudication etc.) or ischemic heart disease. Subjects in this group do not have microalbuminuria. Diabetic cardiovascular group consisted of two subgroups; diabetic hypertensive group (25 patients) and diabetic hypertensive suffering cardiac problems (15 patients).

Other parameters of age (≤60 years), weight, height and BMI were comparable among all groups. Physicians of out-patient's clinic recorded complete clinical examination, medical history and blood pressure.

Smokers and patients suffering urinary tract infection, acute intercurrent infection, chronic analgesic abuse, chronic glucocorticoids treatment and certain chronic diseases as colorectal cancer, hyperthyroidism and Alzheimer disease were excluded.

To provide the diabetic prognostic stages, the patients were classified on the basis of the urinary excretion of albumin: (1) Normoalbuminuric stage (<30 mg/day in a 24 h), (2) microalbuminuria stage (30–299 mg/day), (3) macroalbuminuric stage (>300 mg/day).

Serum glucose, Lipid profile (cholesterol, triglycerides), Serum creatinine and Serum urea were measured using Dade Behring Dimension® clinical chemistry system, UK. The kits were purchased from Dade Behring, Egypt. Haemoglobin A1C was determined by ion-exchange HPLC technique using Bio-Rad D-10 Haemoglobin testing system. C-reactive protein (CRP) was determined by turbidimeteric assay using Spinreact®, S.A.U.

We collect random urine spot sample from each patient for measurement of microalbuminuria (turbidimeteric assays) and Creatinine using ADVIA® 1650 clinical chemistry system, Siemens, Germany to calculate the ACR.

CysC was determined using ELIZA technique. The kit was provided by Biovendor ®, Germany.

Equations for estimating renal function

1. GFR using Cockcroft–Gault equation; CG-GFR = [(140-age in years) X (actual weight in kg) X 0.85 (if female)]/[(72 X serum creatinine in mg/dl)] **(**Cockcroft and Gault, 1976**)**.
2. Cystatin C-based equation; GFR (ml/min) = 99.43 x (cyst C)$^{-1.5837}$ (Larson et al., 2004).

Statistical analysis

A statistical computer program (Graph Pad Instate) was used to test the significance of differences between different groups in the present study. To analyze two sets of data, unpaired Student t-test was used. To analyze more than two sets of data, one way (ANOVA) for parametric data was tried. Correlation analysis was performed using the Spearman's rank test. Statistical significance was defined as the p-value less than 0.05.

RESULTS

The study populations (81 patients: 18 diabetic patients as control, 23 diabetic nephropathy in 2 stages and 40 diabetic CV complications) had a mean age of 50.24±9.7 years. There was no significant difference between the groups in age, BMI or M/F ratio within the group (data not shown).

Microalbuminuria and ACR showed significant increase in patients of nephropathy compared to the control group at (P<0.001). There was also significant increase in Nephropathy stage 1 (DN1) and nephropathy stage 2 (DN2) relative to the control at (P<0.001), whereas there was no significant difference in either microalbuminuria or ACR between diabetic cardiovascular group and the control group **(Figure 1).**

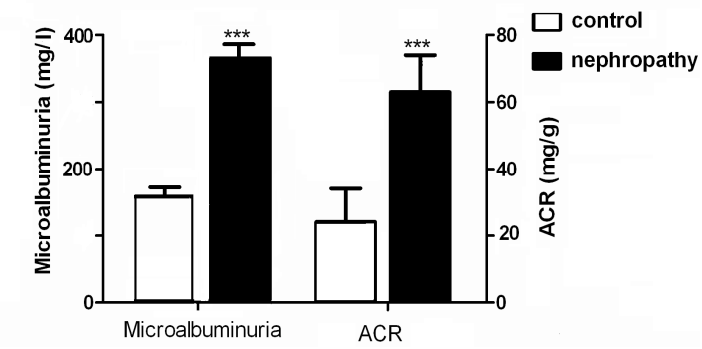


Fig 1: Microalbuminuria and ACR. Significant increase in both microalbuminuria and ACR in nephropathy group compared to diabetic control group at ***P<0.001. The values are expressed as mean±SE. Number of patients (control=18, nephropathy=23).

There was no significant increase in creatinine concentration of stage 1 relative to stage 2 nephropathy, however we found significance increase in stage 1 when compared to the control group (P<0.05), also cardiac diabetics showed no significant increase of their serum creatinine. Serum urea showed no significant increase in stages of nephropathy or diabetic cardiac patients when compared to the control group (data not shown). For lipid profile, it was shown that both cholesterol and triglycerides were different significantly in nephropathy group and cardiac diabetics group in comparison to the control group (P<0.01 and P<0.001 respectively) **(Figure 2).**

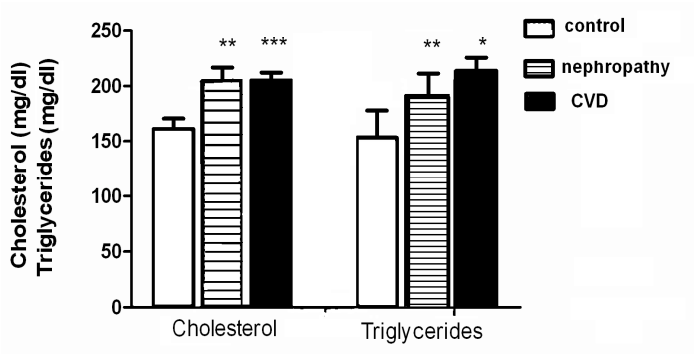
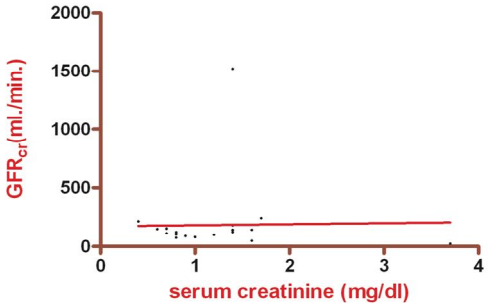


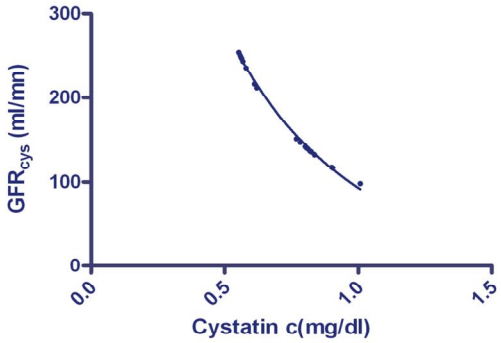
Fig. 2: Lipid profile.
Significant increase in both Cholesterol and Triglycerides in both nephropathy group and CV group compared to diabetic control group at:
***P<0.001 in Cholesterol between CV and diabetic control groups using unpaired t-test with Welch's correction.
****P**<0.01 in Cholesterol and Triglycerides between Nephropathy group and diabetic control groups using unpaired t-test with Welch's correction**.**
*P<0.05 in Triglycerides between CV and diabetic control groups using unpaired t-test with Welch's correction.

Serum creatinine and CysC levels in the study subjects were 0.928±0.44 mg/dL and 0.70±0.26 mg/L, respectively. The comparison of the correlation between CysC-based estimated GFR and creatinine-based GFR measurements (CG-GFR) showed linear correlation in case of CysC (Figure 3a) with no correlation in case of creatinine as shown in Figure 3b. On the basis of the urinary albumin excretion and CG-GFR, the Egyptian type 2 diabetic nephropathy studied patients were divided into 2 stages. Both stages showed significant difference when compared to the control P<0.001. There were no significant differences in sex, age and body mass index. Duration of diabetes (in years) was statistically lower in the control group (2.6±1.7) than in the nephropathy and CV groups (9.26±6.28 and 11±7.26, P<0.0001 respectively). A1C (%), a measure of past 2–3 months, glucose level was lower in the control subjects than in the other groups (data not shown). Serum cystatin C (mg/L) was significantly lower in the control group (0.37±0.06) than in the nephropathy and CV groups (0.72±0.156 and 0.84±0.23 respectively) at P<0.001 as shown in (Figure 4). CRP as progression marker of inflammation showed significant difference between both hypertensive subgroup (HT) and hypertension associated with cardiac problems subgroup (Cp) and the control group (P<0.05). There was no significant difference between cardiovascular subgroups (HT and Cp) at P<0.05 (Figure 5).

CysC, when compared to microalbuminuria, creatinine and CRP, had higher sensitivity and higher specificity reached 100% at the cut off values of our study. Also CysC showed higher PPV, NPV and accuracy which reached 100% (Figure 6).

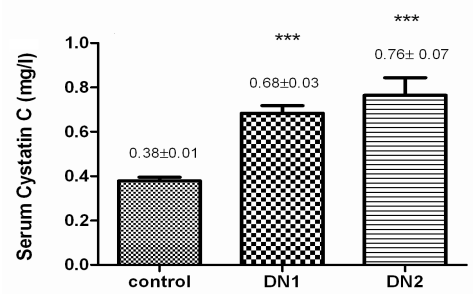


(a) Correlation of GFRcr (ml/min) and serum creatinine. No correlation at P<0.05. Number of patients=23.

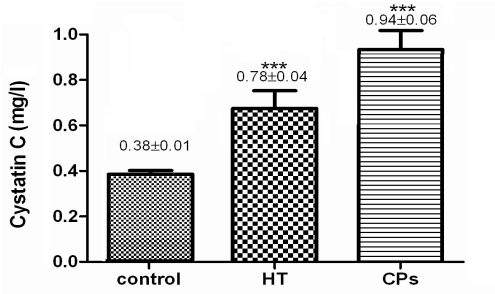


(b) Correlation of GFRCys (ml/min) and serum cystatin c, inversely correlation at P<0.05. Number of patients=23.

Fig 3: correlation of GFR with Serum creatinine and CysC.



(a) Cystatin C showed significant difference between nephropathy group compared to control even in stages 1 and 2 of nephropathy compared to control at P<0.001.



(b) Cystatin C showed significant difference between CV group compared to control even in hypertensive group (HT) and cardiac problem (Cp) compared to control at P<0.001.
Fig 4: Level of CysC in the different groups

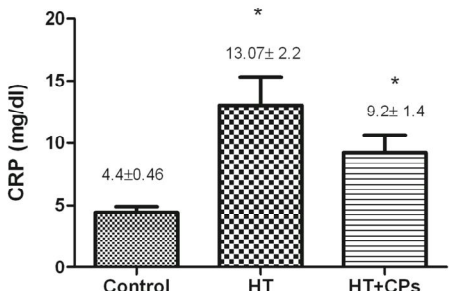
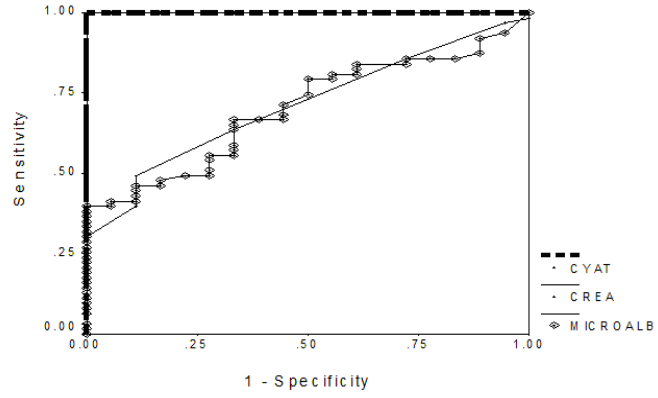
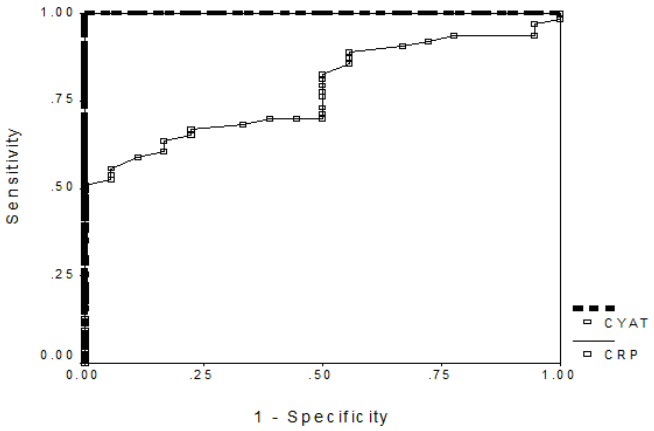


Fig 5: CRP showed significant difference in both hypertensive subgroup (HT) and Hypertensive with cardiovascular problems subgroup (HT+ Cps) when compared to the control group at (P<0.05).



(a) Sensitivity and specificity of CysC compared to Creatinine and Microalbumin.



(b) Sensitivity and specificity of CysC compared to CRP.

Fig 6: superiority of CycC over other markers for nepheropathy.
ROC curve for all markers compared to cystatin C. CystC shows higher sensitivity and specificity than all the other markers. (Number of patients=81).

DISCUSSION

Renal functions assessment is critical for diabetic patients. So there is a need to find a specific and sensitive indicator to identify the early structural and functional changes in diabetic nephropathy. Serum creatinine is considered specific but not very sensitive, as its level does not increase significantly until the GFR is reduced to less than 50% of its normal value (Perrone et al., 1992). In addition, serum creatinine concentration is affected by many factors: age, sex, muscle mass, dietary intake, changes in tubular secretion and various drugs as well as endogenous substances that interfere with its assay, whereas serum CysC may be less affected by those factors. This may explain the possible superiority of serum CysC to serum creatinine for predicting GFR (Perkins et al., 2005).

We have assessed the values of serum CysC or CysC-based GFR in comparison with serum creatinine or creatinine-based GFR to predict diabetic nephropathy in Egyptian patients suffering Diabetes mellitus (type 2). The validation of CysC as a new bioclinical parameter showed a linear relationship (Figure 6) as an indicator of the strong correlation between the tested methods and the reference one. Previous studies showed that CysC-based GFR is more sensitive indicator of decreased GFR than serum creatinine or creatinine-based GFR with variable clinical status (Lee et al., 2005; Lee et al., 2006). Microalbuminuria is not merely a predictor of diabetic nephropathy but also constitutes an evidence of renal damage. This has been demonstrated by our research where the mean of diabetic nephropathy group was above the reference value of 30mg/dl. Also it was different significantly from that of diabetic control patients at P<0.0001. We have found that there was coincidence of microalbuminuria and the presence of glomerular structural lesions, which correlates with recent studies where there were structural lesions in a wide variety of locations in the kidney. This may explain loss of glomerular autoregulation (Mogensen et al., 2003).

Serum level of CysC was increased significantly in all diabetic nephropathy stages compared to the control. In these regards, we may suggest that, in our patients, serum CysC levels might rise earlier than serum creatinine levels in the presence or during progression of type 2 diabetic-nephropathy. Recognizing the decrease in GFR and evaluating the progression stage of diabetic nephropathy, cystatin C-based GFR could be used as a better diagnostic tool than creatinine-based GFR in the absence of additional patient data. Lipoproteins ratio is among the diagnostic tools of cardiovascular complications. The diabetic CV complications include coronary artery disease (CAD), cerebrovascular disease (CVD) and peripheral vascular disease (PVD) (Al-Maskari et al., 2007). We found that LDL/HDL cholesterol ratio exceeded 5.0 in combination with triglycerides greater or equal to 200 mg/dl is a four-fold higher risk for coronary events compared to those with lower LDL/HDL cholesterol ratio and triglycerides below 200mg/dl. Different studies support our results concerning LDL/HDL ratio as risk predictors for CV complications in Diabetes mellitus Type 2 (Panagiotakos et al., 2002).

C-reactive protein (CRP) is a plasma inflammation marker which predicts future coronary events. CRP was increased significantly in diabetic hypertensive patients. Its conc. was more than 6mg/l indicating that hypertension induces inflammation and leads to elevation of CRP level. Also CRP showed significant increase in diabetic hypertensive group, in accord with other studies, which indicated that CRP level may be used as a marker for events involving atherosclerotic plaque rupture and acute

thrombosis more than a marker for events primarily involving progression of lesional stenosis **(**Rifai et al., 2002**).**

CONCLUSION

Cystatin C as a marker of renal functions can facilitate the early prediction of cardiovascular risk along with the other classical risk factors in patients with diabetic CV complications and mild chronic kidney disease.

s

REFERENCES

Al-Maskari F., El-Sadig M., Norman N. The prevalence of macrovascular complications among diabetic patients in the United Arab Emirates. Cardiovascular Diabetology. 2007;6:24- 29.

American Diabetes Association. Standards of medical care in diabetes-2006. Diab. Care. 2006;29:4–42.

Cholongitas E, Shusang V, Marelli L, Nair D, Thomas M, Patch D, Burns A, Sweny P, Burroughs AK. renal function assessment in cirrhosis - difficulties and alternative measurements. Aliment Pharmacol Ther. 2007;26(7):969-78.

Cockcroft D.W., Gault M.H. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16:31–41.

Eriksson P., Deguchi H., Samnegard A., Lundman P., Boquist S., Tornvall P., et al. Human evidence that the cystatin c gene is implicated in focal progression of coronary artery disease. Atheroscler Thromb Vasc Biol 2004; 24:551–557.

Finney H, Newman DJ, Price CP. Adult reference ranges for serum cystatin C, creatinine and predicted creatinine clearance. Ann Clin Biochem. 2000;37 (P1):49-59.

Fujisawa N, Yashiro M, Segawa H, Kadoya Y, Kamata T. Discrepancy between cystatin C and creatinine points leading to a diagnosis of postrenal acute kidney injury and its reversibility: three case reports. Clin Exp Nephrol. 2010;14(6):608-13.

Larson A., Malm J., Grubb A., Hanson L.O. Calculation of glomerular filtration rate express in ml/min from plasma cystatin C values in mg/l. *Chemistry Scand I* Clin Lab Invest. 2004;64:25-30.

Lee S.H., Ahn G.Y., Jeong O.Y., Park Y.J., Jang S.J., Moon D.S. Usefulness of serum cystatin C for the evaluation of renal function in diabetic patient. Korean J. Lab. Med. 2005;25:155–161.

Lee Y.S., Lee K.B., Lhee H.Y., Kim H., Ryu S.H., Bark J.M. Evaluation of glomerular filtration rate by prediction equation in the elderly. Korean J. Nephrol. 2006;25:745–752.

Mogensen C.E. Microalbuminuria and hypertension with focus on type 1 and type 2 diabetes. J Intern Med. 2003;254:45– 66.

Panagiotakos D.B., Pitsavos C., Chrysohoou C., Stefanadis C., Toutouzas P.K. Risk stratification of coronary heart disease. In Greece: Final Results from CARDIO2000 Epidemiological Study. Prev Med. 2002;35:548-556.

Perkins B.A., Nelson R.G., Ostrander B.E., Blouch K.L., Krolewski A.S., Myers B.D., et al. Detection of renal function decline in patients with diabetes and normal or elevated GFR by serial measurements of serum cystatin C concentrations: results of 4- year follow-up study. J. Am. Soc. Nephrol. 2005;16:1404–1412.

Perrone R.D., Madias N.E., Levey A.S. Serum creatinine as an index of renal function: new insights into old concepts. Clin. Chem. 1992;38:1933–1953.

Shlipak M.G., Praught M.L., Sarnak M.J. Update on cystatin C: new insights into the importance of mild kidney dysfunction. Curr. Opin. Nephrol. Hypertens. 2006;15:270–275.

Rifai N., Buring E., Lee I.M., Manson E., Ridker P.M. Is C-Reactive Protein Specific for Vascular Disease in Women? Ann Intern Med., 2002;136:529-533.

Stevens LA, Coresh J, Greene T, Levey AS. Assessing kidney function--measured and estimated glomerular filtration rate. N Engl J Med. 2006;354(23):2473-83.

Vinge E, Lindergård B, Nilsson-Ehle P, Grubb A. Relationships among serum cystatin C, serum creatinine, lean tissue mass and glomerular filtration rate in healthy adults. Scand J Clin Lab Invest. 1999;59(8):587-92.

Visvardis G., Griveas I., Zilidou R., Papadopoulou D., Mitsopoulos E., Kyriklidou P., Manou E., Ginikopoulou E., Meimaridou D., Pavlitou A., Sakellariou G. Transplantation Proceedings (2004);36 (6): 1757-1759.