Research Article

Cystatin C in type 2 diabetic patients in Iraq

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ABSTRACT

Background: Cystatin C (cys-C) is a low molecular mass protein that belongs to the cysteine protease inhibitors; these proteins play an important role in the regulation of proteolytic damage to the cysteine proteases. They have a constant rate of production; its serum concentration is determined by glomerular filtration. Its concentration is not influenced by infections, liver diseases, or inflammatory diseases and used as a marker of glomerular filtration rate. **Materials and Methods:** A total of 107 patients sample (62 males and 45 females) from Al-Hussein Medical City in Karbala Province and Marjan Medical City in Babylon Province and 32 controls (14 males and 18 females) participate in this study. Body mass index (BMI), waist circumference, blood pressure, hemoglobin A1c (HbA1c), microalbuminuria, lipid profile, atherogenic index of plasma, C-reactive protein, and cys-C were measured to all patients and controls. The comparison between control and patients and comparison between albuminuria grades of patients and normoalbuminuria control group were made also the correlation between cys-C and some parameters done. **Results:** The results show significant increase in cys-C in patient males ($P = 0.0001$) and females ($P = 0.03$) as comparison with control group. There was also found an increase in cys-C in all albuminuria grades in males in comparison with normoalbuminuria control, but the increase in females group is found only in microalbuminuria group. There was also significant positive correlation between cys-C and each of BMI, waist circumference, systolic blood pressure, diastolic blood pressure, and HbA1c in male and female groups. **Conclusions:** Cys-C associated with early impairment in the renal function and the elevation appears from normoalbuminuria stage. The kidney function worsens by the presence of obesity, especially central obesity and poor glycemic control.

KEY WORDS: Cystatin-C, Microalbuminuria, Type 2 diabetes

INTRODUCTION

Cystatin-C (cys-C) is a low molecular mass protein that was initially known as inter alia g-trace, post-g-globulin, and gamma-cerebral spinal fluid. The amino acid sequence of the single polypeptide chain of human cys-C was determined in 1981.[1] Cys-C is a non-glycosylated protein that belongs to the cysteine protease inhibitors, cystatin superfamily.[2] These proteins play an important role in the regulation of proteolytic damage to the cysteine proteases. Cys-C is produced at a constant rate by nucleated cells.[3] It is found in relatively high concentrations in many body fluids, especially in the seminal fluid, cerebrospinal fluid, and synovial fluid.[4] 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.[5,6] Therefore, due to its constant rate of production, its serum concentration is determined by glomerular filtration.[7-10] Moreover, its concentration is not influenced by infections, liver diseases, or inflammatory diseases. The use of serum cys-C as a marker of glomerular filtration rate (GFR) is well documented, and some authors have suggested that it may be more accurate than serum creatinine for this purpose.[11-18]

One study for the comparison of serum cystatin C and serum creatinine with GFR in Indian patients with chronic kidney disease (CKD) reveals that serum cystatin C has a high correlation with measured GFR in young and older patients with CKD than creatinine.[19] Other studies on children with early CKD show that cys-C-based equation has a better performance in estimating GFR than creatinine-based equation and that the addition of creatinine equation does not improve the performance of the cys-C-based equation.[20] Serum cys-C is directly related with GFR but not with age and

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is also not influenced by body mass index (BMI) and body surface area.[21] The study aims to evaluate the concentration of cys-C in diabetic patients and compared it with the healthy control group and the usefulness of the use of cys-C test to the microalbuminuria test in detection of early renal impairment.

MATERIALS AND METHODS

Subjects

The samples of this study were taken from diabetic clinic of two hospitals: Al-Hussein Medical City in Karbala Province and Marjan Medical City in Babylon Province. The study included 107 patients: 62 males and 45 females from mentioned hospitals. The control group in this study consists of 32 healthy subjects: 14 males and 18 females. The ages of patients and controls were ranged between 25 and 75 years old.

Methods

The patients and control who have essential hypertension were excluded from the study. The patients who have hypertension before the onset of diabetes were also excluded from the study. The patient was considered to have hypertension dependent on the definition of hypertension: Systolic blood pressure 140 mmHg or more and/or a diastolic blood pressure 90 mmHg or more.[22,23]

BMI:[24,25] The height was recorded to the nearest centimeters and the weight was recorded to the nearest kilograms. BMI was calculated by dividing weight (Kg) by squaring the height (m^2).

$$BMI = \frac{\text{Weight (Kg)}}{\text{Height (m}^2\text{)}}$$

Waist circumference: It was measured at the midpoint between the lower rib and iliac crest with non-stretchable plastic tape. The waist circumference was recorded to the nearest centimeters.[24,25]

C-reactive protein was measured using Turbi-Quick kit and instrument from VITAL diagnostic/Italy Company.

The lipid profile tests were performed using of enzymatic colorimetric procedure using kits from RANDOX/UK Company.

Atherogenic index of plasma (AIP) was calculated as log (triglyceride [TG]/high-density lipoprotein cholesterol).[26,27]

Cys-C was measured by ELISA method using kits from Elabscience/China Company.

Statistical Analysis

Statistical analyses were performed using IBM SPSS statistics software of version 20 and Microsoft Excel 2007. The results were represented as mean ± standard deviation. The analyses of variances were made using independent sample *t*-test and one-way ANOVA.

RESULTS

Subject Characteristics

The subject characteristics for patient and control groups are shown in Table 1. The results of males show that there were significant differences between the control and patient groups in some parameters ($P \leq 0.05$) and this parameter includes age ($P = 0.001$), systolic blood pressure ($P = 0.012$), hemoglobin A1c (HbA1c) ($P = 0.0001$), microalbuminuria ($P = 0.01$), cys-C ($P = 0.0001$), serum TG ($P=0.001$), serum very low-density lipoproteins (VLDL) ($P = 0.001$), and AIP ($P = 0.004$). The results of females show that there were significant differences ($P \leq 0.05$) in

Table 1: Subject characteristics

Parameters	Male control (*n*=31)	Male patients (*n*=62)	*P*-value	Female control (*n*=21)	Female patients (*n*=45)	*P*-value
Age (years)	39.71±13.02	51.79±10.65	0.001	36.71±9.36	48.75±9.23	0.001
BMI (kg/m^2)	27.32±3.96	28.69±3.84	0.12	27.06±6.19	29.37±6.17	0.16
Waist circumference (cm)	96.02±11.73	100.97±12.36	0.07	89.52±15.31	100.36±14.08	0.006
Systolic BP (mmHg)	120.94±11.17	131.94±22.29	0.012	111.29±8.23	125.78±26.13	0.01
Diastolic BP (mmHg)	78.35±8.55	83.00±12.20	0.07	72.24±8.62	79.03±13.24	0.04
Hemoglobin A1c (%)	5.37±1.02	8.20±2.51	<0.001	5.23±0.70	8.64±3.43	0.001
MAU (mg/L)	18.66±38.07	58.06±77.71	0.01	15.51±18.24	77.36±97.09	0.005
C-reactive protein (mg/L)	4.03±3.72	10.55±20.05	0.08	3.08±1.44	14.89±15.46	0.001
Cystatin C (ng/ml)	9.50±11.53	24.41±14.83	0.0001	11.56±13.12	25.32±27.96	0.03
Serum cholesterol (mg/dl)	187.02±49.87	173.04±37.03	0.24	194.55±48.85	203.73±51.15	0.54
Serum triglyceride (mg/dl)	142.36±47.98	231.83±107.22	0.001	134.15±78.45	219.78±101.62	0.004
Serum high-density lipoprotein (mg/dl)	66.48±27.97	59.03±19.43	0.26	59.66±13.29	60.00±33.14	0.96
Serum low-density lipoprotein (mg/dl)	92.07±39.56	73.31±31.34	0.07	108.06±39.64	105.14±44.10	0.82
Serum very low-density lipoproteins(mg/dl)	28.47±9.60	46.37±21.45	0.001	26.83±15.69	43.10±20.32	0.004
Atherogenic index of plasma	0.34±0.21	0.67±0.47	0.004	0.29±0.22	0.73±0.57	0.003

The results represented as mean±standard deviation. The significant value at $P \leq 0.05$. BP: Blood pressure

the parameters: Age (P=0.001), waist circumference (P = 0.006), systolic blood pressure (P = 0.01), diastolic blood pressure (P = 0.04), HbA1c (P = 0.001), microalbuminuria (P = 0.005), C-reactive protein (P=0.001), cys-C (P = 0.03), serum TG (P = 0.004), serum VLDL (P = 0.004), and AIP (P = 0.003).

The results of comparison among different grades of albuminuria in the concentration of cys-C show significant differences between normoalbuminuria control group and three groups of patients in males as shown in Table 2. In females group, the significant differences are found in microalbuminuria group despite it elevated in other groups, but it does not reach to the significant elevations.

The correlation made between cys-C and some parameters revealed some positive linear regression in male and female groups of patients.

Figure 1 shows the positive linear regression between BMI and cys-C in males [Figure 1a] according to the linear equation (y = 1.983 × −33.10) at P = 0.002 and correlation coefficient, r = 0.43 and in females [Figure 1b], according to the linear equation (y = 1.865 × −29.35) at P = 0.005 and correlation coefficient, r = 0.42.

Figure 2 revealed the linear regression between waist circumference and cys-C. In males group [Figure 2a], the positive correlation was not significant at P > 0.05 but in females group [Figure 2b], there was found a significant positive linear regression according to linear equation (y = 0.666 × −41.90) at P = 0.025 and correlation coefficient, r = 0.34.

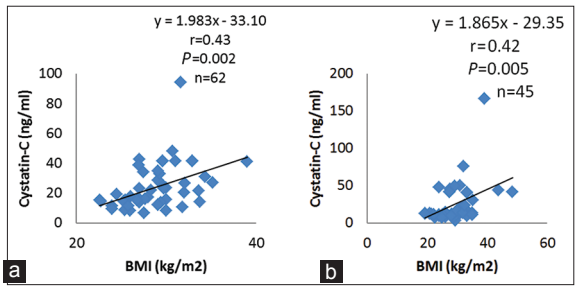

Figure 1: The correlation between body mass index (kg/m^2) and cystatin C (ng/ml). (a) For males group; (b) for females group

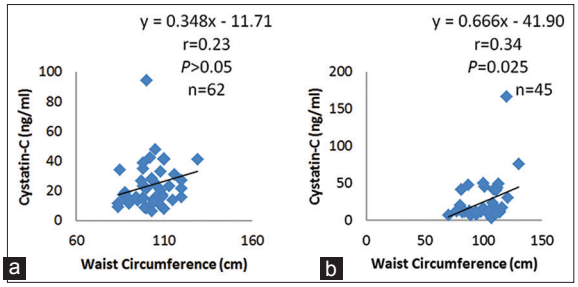

Figure 2: The correlation between waist circumference (cm) and cystatin C (ng/ml). (a) For males group; (b) for females group

Figure 3 shows the correlation between cys-C and systolic blood pressure. There is a significant positive linear regression in males group [Figure 3a] according to linear equation (y = 1.061 × +109.4) at P < 0.001and correlation coefficient, r = 0.64. In females group [Figure 3b], there is also a significant positive linear regression according to linear equation (y = 0.262 × −11.60) at P = 0.011 and r = 0.42.

Figure 4 shows the correlation between cys-C and diastolic blood pressure. In males group [Figure 4a], there is a significant positive linear regression according to the linear equation (y = 0.590 × +68.53) at P = 0.002 and r = 0.58. In females group [Figure 4b], there is a non-significant positive linear regression according to linear equation (y = 0.235 × +74.08) at P > 0.05 and r = 0.30.

The correlation between HbA1c and cys-C is demonstrated in Figure 5. The males group [Figure 5a]

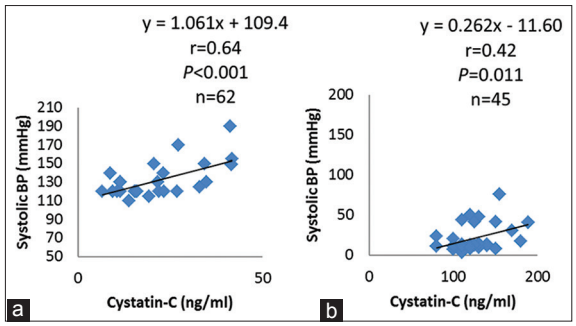

Figure 3: The correlation between systolic blood pressure (mmHg) and cystatin C (ng/ml). (a) For males group; (b) for females group

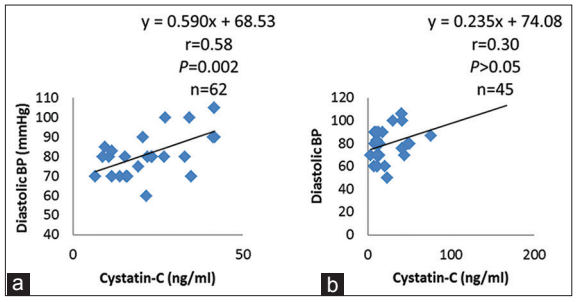

Figure 4: The correlation between diastolic blood pressure (mmHg) and cystatin C (ng/ml). (a) For males group; (b) for females group

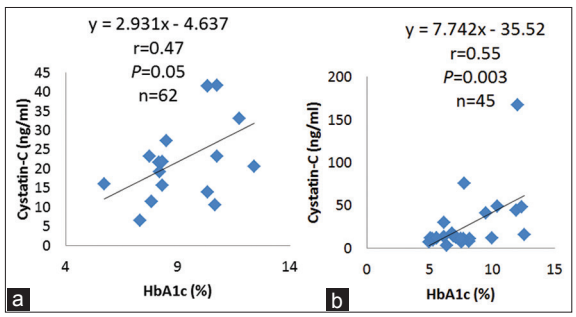

Figure 5: The correlation between hemoglobin A1c (%) and cystatin C (ng/ml). (a) For males group; (b) for females group

Table 2: The comparison among different grades of albuminuria in the concentrations of cystatin C in males and females

Males group		Females group	
Albuminuria grades	**Cystatin C (ng/ml)**	**Albuminuria grades**	**Cystatin C (ng/ml)**
Normoalbuminuria control group (*n*=27)	10.85±11.82	Normoalbuminuria control group (*n*=18)	9.19±10.61
Normoalbuminuria patients group (*n*=34)	24.99±17.48[a]	Normoalbuminuria patients group (*n*=22)	17.85±13.68
Microalbuminuria patients group (*n*=25)	23.52±11.61[b]	Microalbuminuria patients group (*n*=18)	31.93±39.10[b]
Macroalbuminuria patients group (*n*=3)	25.30±8.81[c]	Macroalbuminuria patients group (*n*=5)	28.53±13.66

The results represented as mean±standard deviation. [a]Significant differences in comparison with normoalbuminuria control group, [b]significant differences in comparison with normoalbuminuria patients group, [c]significant differences in comparison with microalbuminuria patients group

demonstrated significant positive linear regression according to linear equation (y = 2.931 × −4.637) at $P = 0.05$ and correlation coefficient, $r = 0.47$.

In females group [Figure 5b], there is a significant positive linear regression according to linear equation (y = 7.742 × −35.52) at $P = 0.003$ and correlation coefficient, $r = 0.55$.

DISCUSSION

Cys-C revealed elevated levels in both males and females in comparison with the control group, as shown in Table 2.

With respect to albuminuria, cys-C elevated from the stage of normoalbuminuria in males and still elevated to macroalbuminuria stage and in females also elevated but shows significantly only in microalbuminuria stage, as shown in Table 2.

These results are consistent with other studies which show that serum cys-C may be used for early prediction for renal function impairment in diabetic patients[28,29] and its levels are useful markers for renal dysfunction in Type 2 diabetic patients with normoalbuminuria[30] and thus, it may be used as supplementary tests to urine albumin excretion to unmask early renal dysfunction.[31]

The results of correlation between cys-C and some parameters revealed a significant correlation with some parameters.

There is a significant positive correlation between cys-C and BMI in both male [Figure 1a] and female [Figure 1b] groups of patients and also between waist circumference and cys-C, but it is positive though it does not reach too significantly in males [Figure 2a] and appears significant positive correlation in females group [Figure 2b]. These results mean that there was a significant correlation between cys-C and obesity in diabetic patients.

One study revealed that there is an association between cys-C and 3-year incident of diabetes but only in people with central obesity or insulin resistance.[32] Other studies also revealed that cys-C production increases more in obese subjects than it does in lean subjects regardless of estimated GFR (eGFR) because the elevated production of cys-C forms adipose tissue in obese subjects[33] and this is related to the status of adipose tissue itself, which includes the enlargement of adipocytes, hypoxia, pro-inflammatory cytokines production, increases number of macrophages, and probably other cellular and molecular alterations known to occur in obesity.[34]

Other studies show that there is a moderate degree of association between cys-C and measures of visceral adiposity as represented by waist-to-height ratio and waist circumference and only a weak association between cys-C and BMI.[35]

The results of correlation between cys-C and blood pressure demonstrate a positive linear regression between cys-C and systolic and diastolic blood pressure in both males and females, as shown in Figures 3 and 4.

These results supported by recent study that shows that there was a positive correlation between cys-C and systolic and diastolic blood pressure regardless of gender, and the correlation had strongest effect on systolic blood pressure, but they did not significantly affect diastolic blood pressure.[36]

The previous study on 24 h ambulatory blood pressure monitoring revealed that systolic blood pressure associated significantly with kidney function, but diastolic blood pressure correlated negatively with cys-C concentrations and cys-C shows a positive relationship with microalbuminuria severity.[37]

The results also show a significant positive correlation between cys-C and HbA1c in both male and female groups of patients, as shown in Figure 5.

This agreement with other studies that found diabetic patients with poor glycemic control (HbA1c >6.5%) had statistically significant higher value of serum cys-C as compared to the good glycemic control.[38] Other studies show that the eGFR corrected by HA1c is considered to be clinically useful and feasible.[39]

CONCLUSIONS

Cys-C associated with early impairment in the renal function and the elevation appears from

normoalbuminuria stage and that makes it more advantageous than albuminuria test in detecting the defects in kidney of diabetic patients. The kidney function is worsen by the presence of obesity, especially the central obesity and poor glycemic control as indicated by the correlation between cys-C and each of BMI, waist circumference, and HbA1c level.

REFERENCES

1. Grubb A, Löfberg H. Human gamma-trace, a basic microprotein: Amino acid sequence and presence in the adenohypophysis. Proc Natl Acad Sci U S A 1982;79:3024-7.
2. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: New insights into old concepts. Clin Chem 1992;38:1933-53.
3. Abrahamson M, Olafsson I, Palsdottir A, Ulvsbäck M, Lundwall A, Jensson O, *et al*. Structure and expression of the human cystatin C gene. Biochem J 1990;268:287-94.
4. Abrahamson M, Barrett AJ, Salvesen G, Grubb A. Isolation of six cysteine proteinase inhibitors from human urine. Their physicochemical and enzyme kinetic properties and concentrations in biological fluids. J Biol Chem 1986;261:11282-9.
5. Grubb A. Diagnostic value of analysis of cystatin C and protein HC in biological fluids. Clin Nephrol 1992;38 Suppl 1:S20-7.
6. Tenstad O, Roald AB, Grubb A, Aukland K. Renal handling of radiolabelled human cystatin C in the rat. Scand J Clin Lab Invest 1996;56:409-14.
7. Pergande M, Jung K. Sandwich enzyme immunoassay of cystatin C in serum with commercially available antibodies. Clin Chem 1993;39:1885-90.
8. Nilsson-Ehle P, Grubb A. New markers for the determination of GFR: Iohexol clearance and cystatin C serum concentration. Kidney Int Suppl 1994;47:S17-9.
9. Kyhse-Andersen J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindström V, *et al*. Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. Clin Chem 1994;40:1921-6.
10. Finney H, Newman DJ, Gruber W, Merle P, Price CP. Initial evaluation of cystatin C measurement by particle-enhanced immunonephelometry on the behring nephelometer systems (BNA, BN II). Clin Chem 1997;43:1016-22.
11. Jung K, Jung M. Cystatin C: A promising marker of glomerular filtration rate to replace creatinine. Nephron 1995;70:370-1.
12. Newman DJ, Thakkar H, Edwards RG, Wilkie M, White T, Grubb AO, *et al*. Serum cystatin C measured by automated immunoassay: A more sensitive marker of changes in GFR than serum creatinine. Kidney Int 1995;47:312-8.
13. Stickle D, Cole B, Hock K, Hruska KA, Scott MG. Correlation of plasma concentrations of cystatin C and creatinine to inulin clearance in a pediatric population. Clin Chem 1998;44:1334-8.
14. Le Bricon T, Thervet E, Benlakehal M, Bousquet B, Legendre C, Erlich D, *et al*. Changes in plasma cystatin C after renal transplantation and acute rejection in adults. Clin Chem 1999;45:2243-9.
15. Randers E, Erlandsen EJ. Serum cystatin C as an endogenous marker of the renal function--a review. Clin Chem Lab Med 1999;37:389-95.
16. Herget-Rosenthal S, Trabold S, Pietruck F, Holtmann M, Philipp T, Kribben A, *et al*. Cystatin C: Efficacy as screening test for reduced glomerular filtration rate. Am J Nephrol 2000;20:97-102.
17. Finney H, Newman DJ, Price CP. Adult reference ranges for serum cystatin C, creatinine and predicted creatinine clearance. Ann Clin Biochem 2000;37:49-59.
18. Risch L, Blumberg A, Huber AR. Assessment of renal function in renal transplant patients using cystatin C. A comparison to other renal function markers and estimates. Ren Fail 2001;23:439-48.
19. Teo BW, Sabanayagam C, Liao J, Toh QC, Saw S, Wong TY, *et al*. Comparison of CKD-EPI cystatin C and creatinine glomerular filtration rate estimation equations in Asian Indians. Int J Nephrol 2014;2014:746497.
20. Hari P, Ramakrishnan L, Gupta R, Kumar R, Bagga A. Cystatin C-based glomerular filtration rate estimating equations in early chronic kidney disease. Indian Pediatr 2014;51:273-7.
21. Kumaresan R, Giri P. A comparison of serum cystatin C and creatinine with glomerular filtration rate in Indian patients with chronic kidney disease. Oman Med J 2011;26:421-5.
22. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, *et al*. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. Hypertension 2003;42:1206-52.
23. Weber MA, Schiffrin EL, White WB, Mann S, Lindholm LH, Kenerson JG, *et al*. Clinical practice guidelines for the management of hypertension in the community: A statement by the American society of hypertension and the international society of hypertension. J Clin Hypertens (Greenwich) 2014;16:14-26.
24. Shah A, Bhandary S, Malik SL, Risal P, Koju R. Waist circumference and waist-hip ratio as predictors of Type 2 diabetes mellitus in the nepalese population of Kavre district. Nepal Med Coll J 2009;11:261-7.
25. Akinola OB, Omotoso OG, Akinlolu AA, Ayangbemi KD. Identification of the anthropometric index that best correlates with fasting blood glucose and BMI in post-pubescent female Nigerians. Anat J Afr 2014;3:324-8.
26. Dobiásová M. Atherogenic index of plasma [log(triglycerides/HDL-cholesterol)]: Theoretical and practical implications. Clin Chem 2004;50:1113-5.
27. Nwagha UI, Ikekpeazu EJ, Ejezie FE, Neboh EE, Maduka IC. Atherogenic index of plasma as useful predictor of cardiovascular risk among postmenopausal women in Enugu, Nigeria. Afr Health Sci 2010;10:248-52.
28. El-Kafrawy N, Shohaib A, Barbary H, Seleem A. Evaluation of serum cystatin C as an indicator of early renal function decline in Type 2 diabetes. Menoufia Med J 2014;27:60.
29. Shima T, Khatun A, Yeasmin F, Ferdousi S, Kirtania K, Sultana N. Cystatin C: A better predictor of kidney function in diabetic patients. Bangladesh J Med Biochem 2013;4:16-20.
30. Jeon YK, Kim MR, Huh JE, Mok JY, Song SH, Kim SS, *et al*. Cystatin C as an early biomarker of nephropathy in patients with Type 2 diabetes. J Korean Med Sci 2011;26:258-63.
31. Papadopoulou-Marketou N, Skevaki C, Kosteria I, Peppa M, Chrousos GP, Papassotiriou I, *et al*. NGAL and cystatin C: Two possible early markers of diabetic nephropathy in young patients with Type 1 diabetes mellitus: One year follow up. Hormones (Athens) 2015;14:232-40.
32. Reutens AT, Bonnet F, Lantieri O, Roussel R, Balkau B, Epidemiological Study on the Insulin Resistance Syndrome Study Group, *et al*. The association between cystatin C and incident Type 2 diabetes is related to central adiposity. Nephrol Dial Transplant 2013;28:1820-9.
33. Guerre-Millo M. Serum cystatin C concentrations are increased in human obesity in relation to over-production by the adipose tissue. Obesity (Silver Spring) 2012;20:1755.
34. Taleb S, Cancello R, Clément K, Lacasa D. Cathepsin s promotes human preadipocyte differentiation: Possible involvement of fibronectin degradation. Endocrinology 2006;147:4950-9.
35. Panaich SS, Veeranna V, Bavishi C, Zalawadiya SK, Kottam A, Afonso L, *et al*. Association of cystatin C with measures of obesity and its impact on cardiovascular events among healthy US adults. Metab Syndr Relat Disord 2014;12:472-6.
36. Chen H, Wang T, Yang Q, Tang S. Correlation of serum cystatin C with blood pressure: A cross-sectional study of 912 subjects. Nan Fang Yi Ke Da Xue Xue Bao 2015;35:1055-8.
37. Mena C, Robles NR, de Prado JM, Gallego FG, Cidoncha A.

Cystatin C and blood pressure: Results of 24 h ambulatory blood pressure monitoring. Eur J Intern Med 2010;21:185-90.
38. Senghor A, William E, Naveen C, Krishnan S. Correlation of Cystatin C and cardiovascular risk markers in uncontrolled Type 2 Dm. Int J Pharm Clin Res 2013;5:79-82.
39. Tsuda A, Ishimura E, Ohno Y, Ichii M, Nakatani S, Machida Y, *et al*. Poor glycemic control is a major factor in the overestimation of glomerular filtration rate in diabetic patients. Diabetes Care 2014;37:596-603.

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