ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Diagnostic Values Of Serum Cystatin C And Urinary Fetuin-A As Early Biochemical Markers In Predicting Diabetic Nephropathy Among Patients With Type 2 Diabetes Mellitus.

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ABSTRACT

Diabetes accounts for 30% to 50% of the incident cases of end-stage kidney disease in the United States and also in Egypt is the second leading cause of end stage renal disease after hypertension. The current study aimed to determine the validity of urinary fetuin-A and serum cystatin C as early diagnostic biomarkers of diabetic kidney disease in type 2 diabetes mellitus (T2DM). Fifty patients with type 2 diabetes mellitus were categorized into two subgroups, group I noromoalbuminuric diabetics [with normal albumin/creatinine ratio (A/C ratio)], group II microalbuminuric diabetics (with A/C ratio more than 30 mg Alb/ g creat) compared to 20 apparently healthy non diabetic individuals with matched age (group III). Serum cystatin C and urinary Fetuin-A were measured in addition to albumin/ creatinine ratio and creatinine. Estimated glomerular filteration rate (eGFR) has been calculated. Overall, there were significant elevations of both serum cystatin C and urinary fetuin –A in microalbuminuric diabetic patients than noromoalbuminuric diabetic patients (p value =0.0001). There were significant positive correlations between serum cystatin C and urinary Fetuin-A in both normoalbuminuric and microalbuminuric diabetic patients ($p<0.05$ for all). Serum cystatin C at cut-off point> >1.6 mg/ml showed 96% sensitivity , 94% specificity, while for urinary feutin-A (at cut-off point> 120 mg/L) were 98%, 96% respectively, in predicting diabetic nephropathy among T2DM. Both serum cystatin C and urinary Fetuin-A can be used as early biomarkers for diabetic nephropathy in T2DM.

Keywords: Cystatin-C, Fetuin-A, biomarkers, Diabetic nephropathy, T2DM.

https://doi.org/10.33887/rjpbcs/2019.10.6.29

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ISSN: 0975-8585

INTRODUCTION

The chronic hyperglycemia of diabetes mellitus is associated with relatively specific long-term microvascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular disease [1,2]. Diabetic kidney disease and diabetic nephropathy are the leading cause of end-stage kidney disease in the United States and most developed countries [3]. Diabetic nephropathy is a principal cause of morbidity and has an association with increased cardiovascular mortality in T2DM [4]. The natural history of diabetic kidney disease includes glomerular hyperfiltration, progressive albuminuria, declining GFR, and ultimately, end stage renal disease (ESRD) [5]. The prevalence of diabetic nephropathy among ESRD patients in Egypt increased from 8.9% in 1996 to 14.5% in 2001[6].

Serum cystatin C may be used for screening patients with poorly controlled diabetes mellitus or hypertension when serum creatinine level is inconclusive [7] It has been hypothesized that the serum Cystatin C level is a superior marker of the glomerular filtration rate (GFR) to the serum creatinine level [8].Most diabetic subjects with elevated cystatin C had normal serum creatinine levels, normoalbuminuria and reduced GFR. Serum cystatin C may be considered as an early marker than both microalbuminuria and serum creatinine [9].

Fetuin-A [also referred to as α-2 HeremansSchmid glycoprotein (AHSG)] is a multifunctional glycoprotein which is exclusively secreted from the hepatocytes in human became pronounced that it could inhibits insulin receptor substrate-1 and stimulated a lower-grade inflammation, which led to insulin resistance in T2DM [10]. Higher excretion of Fetuin-A into urine has been reported to reflect the increase in insulin resistance and inflammatory responses in obesity and type 2 diabetes [11]. Thus the present study has been designed to validate the possible use of cystatin-C and urinary feutin-A as predictor markers for diabetic nephropathy among T2DM. Also to evaluate the correlations between urinary fetuin-A, serum cystatin C with microalbuminuria in such patients.

MATERIALS AND METHODS

Study design and participants

A cross-sectional study included 50 patients with T2DM recruited from the outpatients nephrology clinics of Abo-Tesht Central Hospital, Qena, Egypt between January 2017 and January 2019. They were categorized into group I (included 30 patients with T2DM with normal albumin / creatinine ratio, 18 males and 12 females, aged from 30 years to 70 years with mean value 47.90 years±9.11 SD), and group II(20 T2DM patients with microalbuminuria, 13 males and 7 females, aged from 30 years to 70 years with mean value 48.86years±11.02 SD). Additionally, ,20 apparently healthy non diabetic individuals (12 males and 8 females) with matched age were included as a control group(Group III). The current study has been done in accordance with the Declaration of Helsinki and after approval of the Ethics Committee of Assiut University Hospitals, Egypt. An informed written consent has been obtained from every included subject. Those with urinary tract infections, malignancies, liver diseases, thyroid dysfunction, congestive heart failure or hypertension were excluded from the study.

Biochemical assays

Urine microalbumin concentration was determined by microalbumin-turbilatex latex turbidimetry quantitative determination of microalbumin, then albumin to creatinine ratio was calculated from the equation: Urine microalbumin / Urine creatinine x 100 mg/g creatinine where the equation takes urine albumen in mg/L, and urine creatinine in mg/dl. Estimated GFR has been calculated from the MDRD equation: $186 \times (\text{Creatinine}/88.4)^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$.

Serum cystatin C concentration was measured via nephlometric technique using commercially available cystatin C kits supplied by:AGAPPE diagnostics LTD, MispAi2, India with catalog number 12009005. Urinary human Fetuin-A concentration was determined using commercially available ELISA assay kit supplied by Bioassay Technology Laboratory with catalog number E1386Hu, using microplate ELISA reader (EMR-500, USA).

Statistics

ISSN: 0975-8585

The Statistical Package for Social Sciences (SPSS) software program (version 20; Armonk, NY:IBM Corp) was used for the data analysis. Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test. Quantitative data were described using mean and standard deviation for normally distributed data while abnormally distributed data was expressed using median, minimum and maximum. For normally distributed data, comparison between two independent populations were done using independent t-test while more than two populations were analyzed F-test (ANOVA) to be used. Pearson correlation was done to measure the correlation between quantitative variables in case of parametric data. Medcalc Program was used to calculate sensitivity, specificity, positive and negative predictive values. P-value considered statistically significant when P <0.05.

RESULTS

The mean serum creatinine, A/C ratio, serum cystatin-C and urinary feutin-A were significantly higher among normoalbuminuric and microalbuminuric T2DM when compared with the controls (p<0.05) and eGFR was significantly lower among the patients subgroups vs. controls (p<0.05) as presented in (Table.1).

Table 1. Comparison between the studied groups regarding s. creatinine, eGFR, A/C ratio, s. cystatin C, and urinary fetuin-A

Biochemical parameters		Studied groups			P values		
		Group I "T2DM with normal A/C ratio" n=30	Group II "T2DM with A/C ratio > 30" n=20	Group III "Control" n= 20	P1 comparison between group I and II	P2 comparison between group I and III	P3 comparison between group II and III
S. Creatinine (mg/Dl)	Range Mean SD	0.8-1.35 1.06 0.15	0.95-5.2 1.89 0.99	0.6-1.0 0.80 0.13	0.001*	0.001*	0.003*
eGFR (ml/min/1.73m^2)	Range Mean SD	48-100 72.71 13.78	12-94 48.72 18.37	78.5-131 103.14 12.36	0.0001*	0.0001*	0.0001*
A/C ratio mg/g creatinine	Range Mean SD	11.2-29 22.12 4.79	75-620 204.05 138.18	6-24 14.02 5.27	0.0001*	0.0001*	0.0001*
S. Cystatin C mg/dL	Range Mean SD	0.8-1.4 1.06 0.18	1.22-4.95 1.99 1.03	0.68-1 0.80 0.09	0.0001*	0.0001*	0.0001*
U. Fetuin-A mg/L	Range Mean SD	14-34 24.9 4.82	99-854 320.09 199.97	7.8-27 14.27 6.46	0.0001*	0.0001*	0.0001*

N.B: P value>0.05 insignificant, * P<0.05 significant.

There were significant positive correlations between serum cystatin-C and urinary feutin-A among groupI and II , p<0.05. Additionally, there were significant positive correlation between A/C ratio and urinary feutin-A and significant negative correlation was confirmed between urinary feutin-A and eGFR among group II , p<0.05 as presented in (Figure 1A,1B, 1C and 1D).

ISSN: 0975-8585

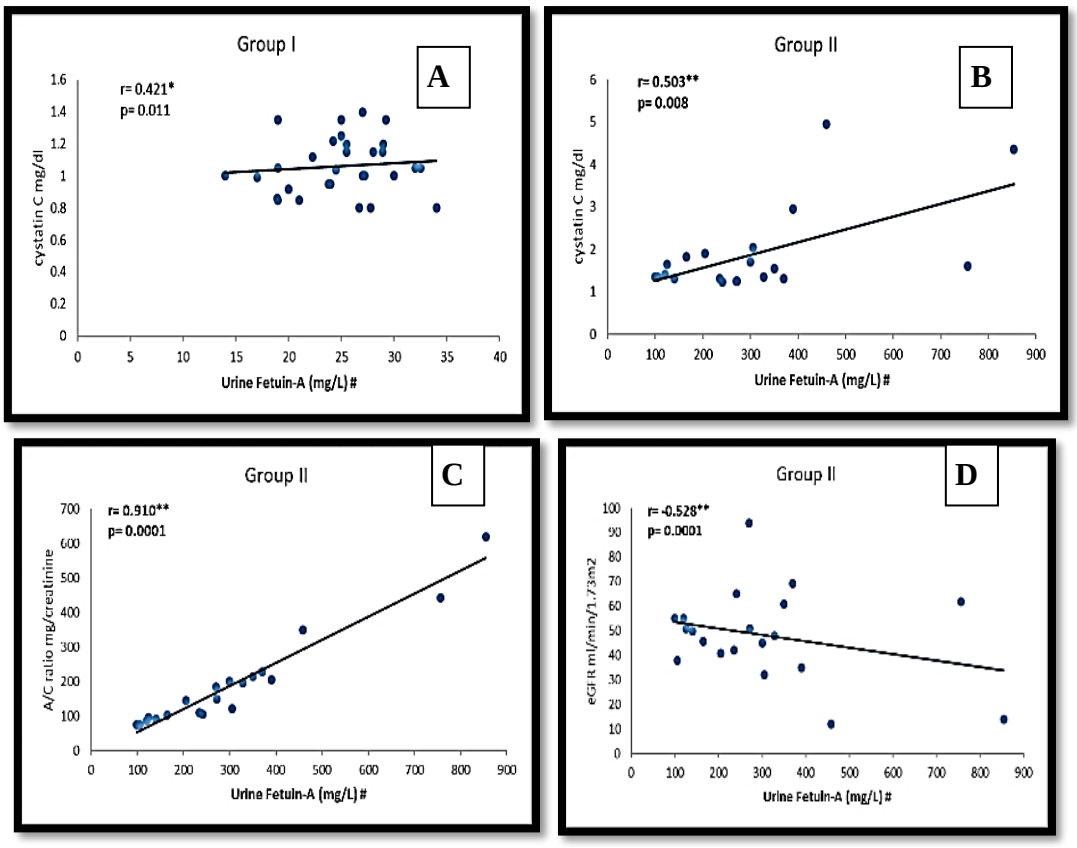

Figure1: Significant positive correlations between serum cystatin-C and urinary feutin-A among groupI (A) and II (B). Significant positive correlation was found between A/C ratio and urinary feutin-A among group II (C). Significant negative correlation was confirmed between urinary feutin-A and eGFR among group II (D).

Serum cystatin C at cut-off point> >1.6 mg/ml showed 96% sensitivity , 94% specificity, while for urinary feutin-A (at cut-off point> 120 mg/L) were 98%, 96% respectively, in predicting diabetic nephropathy among T2DM (Table 2, Figure 2).

Table 2: Cut off value, sensitivity, specificity and accuracy of urinary feutin-A and cystatin-C in predicting diabetic nephropathy among T2DM.

Biochemical markers	Area	Cut off value	Sensitivity	Specificity	Accuracy	Asymptotic 95% C.I.	
						Lower Bound	Upper Bound
Urinary fetuin-A (mg/L)	.991	>120.0	98.0	96.0	97.0	.946	1.000
S. cystatin C (mg/dl)	.966	>1.6	96.0	94.0	95.0	.929	1.000

ISSN: 0975-8585

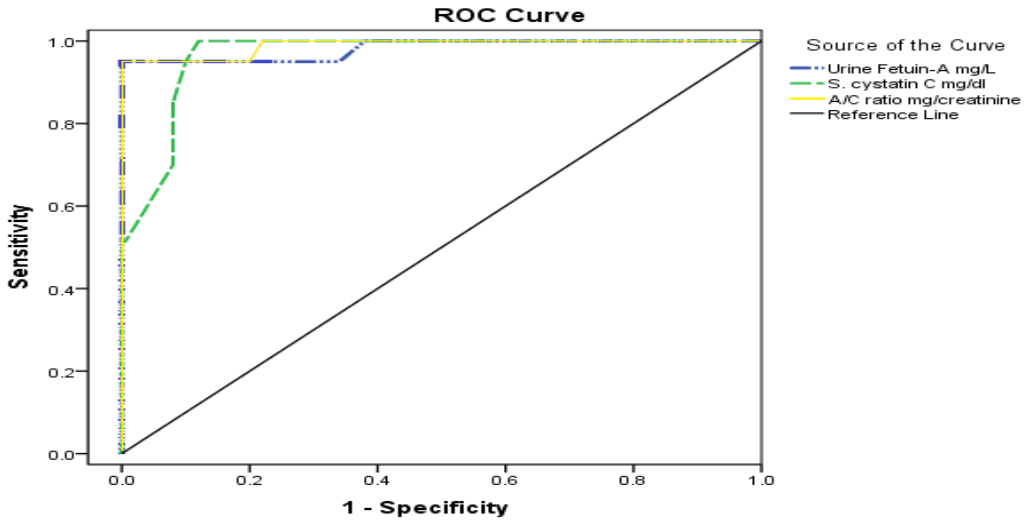

Figure 2: Receiver Operating Characteristic (ROC) curves for serum cyctatin-C and urinary feutin-A in diagnosing diabetic nephropathy among T2DM patients using A/C ratio as a standard parameter.

DISCUSSION

Regarding serum cystatin C in our study there was significant rise of their serum levels in T2DM patients with normal A/C ratio (group I), compared with its level in the apparently healthy control group (group III), and its level was significantly much higher in T2DM with nephropathy (group II). Additionally, we found that in group II , a significant positive correlation between serum level of cystatin c and A/C ratio and there was a significant negative correlation between level of serum cystatin C and eGFR , but in group I we find no significant correlations between cystatin c and A/C ratio or eGFR as these parameters were normal but serum cystatin C was raised in this group (group I), and these results were in line with Jeon et al.[12], who reported that serum and urinary cystatin C levels were increased with increased degree of albuminuria, reaching higher levels in macroalbuminuric patients. Especially, in normoalbuminuric patients, serum and urine cystatin C were identified as independent factors associated with eGFR< 60 mL/min/1.73 m^2 estimated by MDRD equation. The cystatin C levels of serum and urine could be useful markers for renal dysfunction in type 2 diabetic patients with normoalbuminuria.

Our study by these findings coincide with Jeon et al.[13], as their study reported that cystatin C showed a performance similar to that of serum creatinine, in addition, cystatin C levels increased with increasing CKD stage I to III and from normo to microalbuminuria and showed a positive correlation with A/C ratio. In a comparison of renal function markers in diabetic patients according to serum Cystatin C level, all markers including A/C ratio, serum creatinine, and eGFR showed significant differences between patients with cystatin C level and their study concluded that serum cystatin C is a useful marker of early renal impairment in type 2 diabetic patients because it reflects both a decrease in GFR and elevated A/C ratio.

Our study also agrees with El-Kafrawy et al.[14], they said that we found a significantly higher concentration of serum creatinine and serum cystatin C in the macroalbuminuric group compared with the normoalbuminuric and microalbuminuric groups (diabetics) , and all groups had higher levels compared with the control group (Non diabetics).Our findings also coincides with Fiseha et al.[15], who concluded that although microalbuminuria has been recognized as the earliest marker for detection and prediction of diabetic nephropathy (DN), it has several limitations, such as lower sensitivity and larger variability, cystatin C being elevated in serum or urine, even before the appearance of albuminuria and creatinine based estimates (eGFR), cystatin C might offer an advantage to traditional CKD markers with respect to early detection of DN and its progression, which will allow for timely intervention and management of type 2 DN. Thus, their study demonstrates that cystatin C offers a more efficient diagnostic tool than traditional CKD markers in type 2 diabetic patients with renal disease.

Our study also coincides with Ashwin Kumar et al.[16], they said that serum creatinine as well as serum cystatin C levels were significantly elevated in the study group (diabetics) as compared to non-diabetic

controls. There was a strong positive correlation of serum cystatin C with serum creatinine. Additionally, our study coincides with Brijesh and Saurav [17], they said that serum cystatin levels were increased significantly in diabetics with history of more than 10 years and is earlier marker of DN in comparison to serum creatinine. The increase in levels correlates well with increase in microalbumin levels in urine in early stages of DN. And our study coincides with Konsouh et al.[18], they concluded that microalbuminuric diabetic patients showed increased serum cystatin c, and the severity of renal damage caused by diabetic disease is well reflected by these levels. In addition, there was significant negative correlation between serum cystatin c and estimated glomerular filtration rate. Serum cystatin c measurement might become a useful, practical and noninvasive accurate tool for early detection of microalbuminuria and renal insufficiency. Our study also coincides with Sim et al. [19], they said that serum cystatin C levels were significantly increased in subjects with diabetic conditions compared to in those with normal glucose levels and higher levels of serum cystatin C were associated with an increased prevalence of diabetic conditions. Many researchers were in accordance with our data regarding cyctatin-C [9, 20-24].

Regarding the rule of urinary Fetuin –A in diabetic nephropathy there is a shortage in research in this issue worldwide but a few of studies present and to the best of our knowledge there is no previous studies concerning of urinary fetuin –A in diabetic nephropathy in Egypt, but most of the studies was in serum fetuin-A in diabetic nephropathy as Al-Said et al [10], who concluded that, fetuin-A levels were significantly higher in all T2DM groups compared with controls and they reported that fetuin-A may be used as a marker for microvascular complications in T2DM, especially the diabetic nephropathy. Antifetuin drugs may be invented to delay diabetic microvascular complications. Also there is for study of assessment of urinary fetuin-A level of patients suffered with urolithiasis as Arora et al.[25], they reported that The patients with bilateral, multiple, and recurrent renal calculi had significantly lower 24-h urine Fetuin and serum Fetuin adjusted to serum creatinine values compared with individuals without stones. Serum and urine fetuin evaluation may be included in the metabolic profile of stone formers with a high risk of recurrence. Mehrsai et al. [26] and Kumar et al.[27], both reported that serum and urinary fetuin-A levels of patients with kidney stones were significantly lower than in healthy individuals and based on multivariate logistic regression analysis, urinary fetuin-A levels were inversely associated with the risk of kidney stones. Fetuin is also an important marker of renal damage and higher serum fetuin-A values have been found in end-stage renal failure in various prior publications.

Results of our study coincide with Inoue et al. [28], who reported that a higher urinary Fetuin-A excretion demonstrated a higher risk for the development of microalbuminuria and reduction of renal function. Higher excretion of Fetuin-A into urine has been reported to reflect the insulin resistance and inflammatory responses in obesity and type 2 diabetes [11]. Urinary excretion of Fetuin-A is a candidate for the biomarker to predict the progression of diabetic nephropathy [29]. It may reflect the increase in the serum levels of Fetuin-A and alterations in the changes in the permeability of glomerular capillaries. Fetuin-A is reported to pass through the slit diaphragm and re-introduced to proximal tubular cells by megalin-mediated endocytosis [30].

Our findings revealed that serum cystatin C at cut-off point> >1.6 mg/ml showed 96% sensitivity , 94% specificity, while for urinary feutin-A (at cut-off point> 120 mg/L) were 98%, 96% respectively, in predicting diabetic nephropathy among T2DM.

CONCLUSION

This study was performed on upper Egyptian patients suffering type 2 diabetes mellitus and its results concluded that there is a significant rise in both serum cystatin C and urinary fetuin-A in all diabetics even before appearance of albumin in their urine and become much higher in diabetics with albuminuria So, both of serum cystatin C and urinary fetuin-A can be used as early biomarkers for diabetic nephropathy in type 2 diabetics.

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