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FETUIN-A IN PATIENTS WITH KIDNEY DISEASE

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ABSTRACT

Urinary exosomes containing apical membrane and intracellular fluid are normally secreted into the urine from all nephron segments and may carry protein (like Fetuin-A) a marker of renal dysfunction and structural injury. This study was designed to assess the use of urinary fetuin -A biomarker in patients with chronic kidney disease (CKD). A study was done on 40 patients (25 males and 15 females), aged (62 years ±8) with chronic kidney disease and 30 healthy subjects (12 males and 18 females), aged (60 years± 6). Renal function tests, lipid profile and urinary levels of fetuin-A and α_1 –microglobulin were measured and studied. The statistical results of urinary fetuin –A and α_1 –microglobulin levels showed high significant increases ($P< 0.001$) in patients with chronic kidney disease versus control subjects (1.32 ng/gcr. ±0.88 *vs.* 0.34 ng/gcr. ±0.28), (15.03µg/gcr.±18.05 *vs.* 3.5 µg/gcr.±2.7) respectively. Moreover, the urinary fetuin–A levels increase 3.88 folded in chronic kidney disease patients compared with control healthy subjects. Therefore urinary Fetuin-A may be used to predict the progression of renal injury.

KEYWORDS: Chronic kidney disease, fetuin-A, α_1 -Microglobulin.

1. INTRODUCTION

Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function, present for more than 3 months and is classified based on cause, glomerular filtration rate (GFR) category and albuminuria category.[14] Urine is an ideal non-invasive source of biomarkers to diagnose and classify kidney diseases. However, conventional urine markers (casts, fractional excretion of sodium) are non specific and in sensitive.[13]

Many biomarkers used in the detection of incipient nephropathy and risk assessment of cardiovascular disease; such as transferrin, type IV collagen and N-acetyl-b-D-

glucosaminidase, inflammatory markers including tumour necrosis factor-α, transforming growth factor-β, vascular endothelial growth factor and monocyte chemoattractant protein-1, as well as oxidative stress markers such as 8-hydroxy- 2´ - deoxyguanosine. However, the sensitivity of these markers compared with albumin requires further investigation.[1]

Moreover, exosomes were isolated from human urine by differential centrifugation and demonstrated the presence of several disease-related proteins.[10,11] Exosomes are nanovesicles containing vesicular membranes and intracellular fluid are normally secreted in to the urine from all nephron segments, and contain proteins that may be altered in abundance or physical properties in association with various renal diseases.[12]

Nevertheless, many important functions, including exchange of DNA, mRNA and micro RNA have been suggested for these nanovesicles.[5,6] Many studies have suggested their involvement in the maintenance of anchorage of independent growth, adhesion, cellular motility and invasion.[7,8]

Fetuin-A is a serum glycoprotein synthesized and secreted mainly by the liver parenchymal cells and to a lesser extent, kidney, brain and testis cells.[2,3] Its role in cellular adhesion has been debated for many years mainly because it co-purifies with a number of plasma proteins including fibronectin and α_2- macroglobulin.[4]

1.1 PATIENTS AND METHODS

Sixty Forty patients, (15 females, 25 males), aged (62 years ±8) with chronic kidney disease and 30 control subjects (18 females, 12 males) aged (60 years ±6) recruited from Al-yarmouk hospital. Diagnoses are made based on clinical symptoms and biochemical tests. Patients with liver disease, renal failure, heart failure and patients with eGFR 15 ml/min/1.73m^2 or under dialysis were excluded from the current study.

Blood samples are aspirated to measure HbA1c % and serum levels of total protein, albumin, creatinine, urea, uric acid and lipid profile by Photometric Colorimetric Test. Exosomes are isolated from urinary sample using differential centrifugation. The first few steps in the isolation of exosomes involves removal of dead cells and cell debris in which samples are first centrifuged at 300xg to remove the cells followed by centrifugation at 3000xg for 10 min and 10,000 xg for 30 min to remove larger and smaller cells debris respectively. Second step involves centrifugation of sample at 100,000xg for 70 min to pellet the exosomes fraction

followed by washing the pellet in phosphate buffered saline (PBS) and centrifugation at 100,000xg for 70 min. Finally, the pellet is dissolved in required amount of PBS.[23]

Urinary levels of fetuin-A and α_1-Microglobulin assayed by the quantitative sandwich enzyme immuno- assay technique (ELISA).Estimated glomerular filtration rate (eGFR) was calculated using 2009 CKD-EPI creatinin equation by equation.[15]

All blood and urine samples were obtained after receiving patients' informed consent and followed a standardized protocol that approved by the institutional ethics committee. Results are shown as mean ± SD with 95% confidence interval (CI) and *P* values of $0 < 0.05$ were regarded to be statistical significant. All statistical analyses were performed using series SPSS version 16.

1.1.1 RESULT

The statistical results presented in (Table -1) showed high significant decrease ($P<0.001$) in mean serum levels of total protein, albumin and lipid profile except HDL-cholesterol in patients with chronic kidney disease as compared with control healthy subjects.

Whereas high significant increase ($P<0.001$) noted in mean serum levels of creatinine, urea and uric acid and in mean urinary levels of fetuin –A and α_1-Microglobulin in patients with chronic kidney disease as relation to control mean, (Figure-1and Figure-2).

Table 1. Clinical and biochemical characteristics of the studied groups.

	Control	CKD	P-value
Number (Male/Female)	30(12/18)	40(25/15)	
Age (years)	60± 6	62±8	
BMI (Kg/m^2)	27.5±6.5	23.5±5.5	**
HbA1C %	7.4 %±0.65	6.8%±0.9	
Serum Total protein (g/dl)	7.55±0.57	5.6± 0.54	***
Serum Albumine (g/dl)	4.26± 0.56	3.35± 0.55	***
Serum Creatinine (mg/dl)	0.74±0.3	1.67 ±0.8	***
Serum Urea (mg/dl)	32.25±12.4	70.6±25.7	***
SerumUric acid (mg/dl)	5.2±0.67	7.2 ±1.5	***
Serum Total Cholesterol (mg/dl)	187.6±15.4	175.23±30.7	***
Serum Triglyceride (mg/dl)	178.25 ±20.4	163.83±26.6	***
Serum HDL-C (mg/dl)	50.8 ±10.03	52.5±15.5	
Serum LDL-C (mg/dl)	102.64±25.7	92.25±30.19	***
eGFR (ml/min)	98.2±14.67	45.6 ±15.33	***
Urinary Fetuin –A (ng/gcr.)	0.34±0.28	1.32±0.88	***
Urinary α_1-Microglobulin (µg/gcr.)	3.5±2.7	15.03±18.05	***

BMI=body mass index; CKD=chronic kidney disease patients; eGFR=estimated glomerular filtration rate;

*** =high significant difference P<0.001; ** = high significant difference P<0.005.

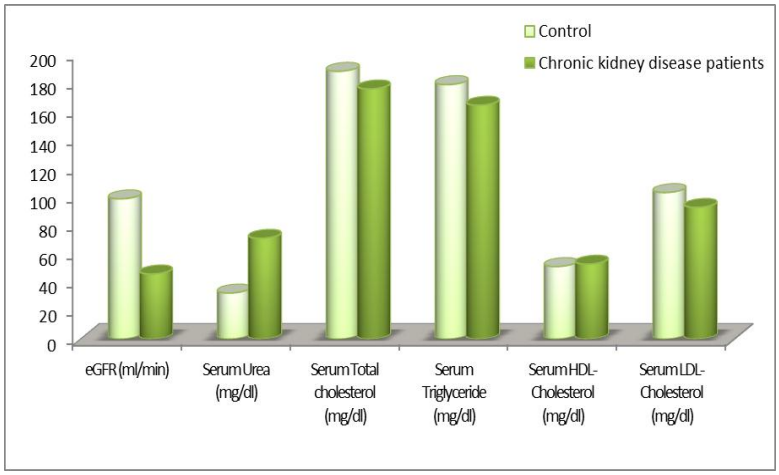

Figure -1: The mean serum levels of total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, urea and estimated glomerular filtration rate for control subjects and for chronic kidney disease patients.

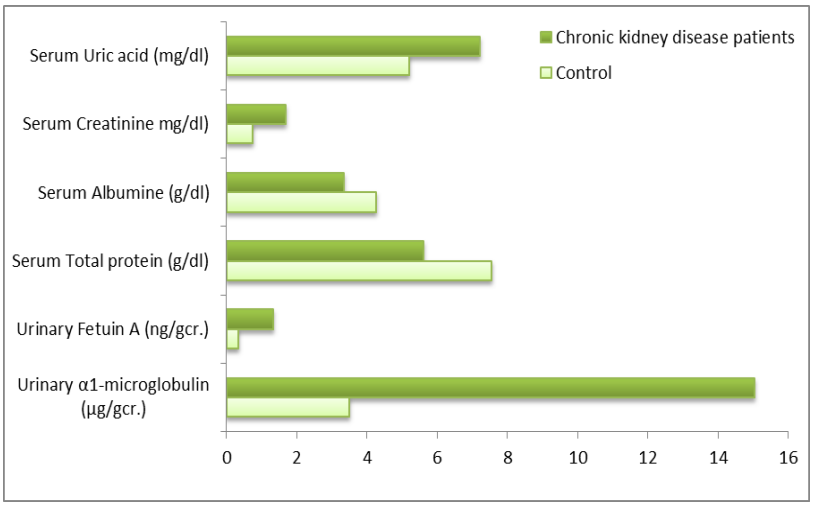

Figure -2: The mean serum levels of total protein, albumine, creatinine and uric acid and the mean urinary levels of fetuin –A and α_1-microglobulin for control subjects and for chronic kidney disease patients.

1.1.2 DISCUSSION

Fetuin-A is synthesized in liver and secreted into the blood stream, where it is a negative acute- phase response protein and plays its anti-inflammatory role via suppressing release of tumor necrosis factor -alpha induced by lipopolysaccharide in vitro and vivo.[17]

Fetuin-A is also a systemically acting inhibitor of ectopic calcification.[18] High plasma Fetuin-A levels are correlated with insulin resistance and fatty liver in human.[21] and are also independently associated with metabolic syndrome in non-diabetic patients with coronary artery disease.[22] Furthermore, decreased serum Fetuin-A concentration is associated with a higher mortality rate in dialysis patients and can predict mortality of chronic kidney diseases.[19,20] Although exosomal Fetuin-A may be synthesized by the kidney, it may also appear in the urine as a result of incomplete proximal tubule processing in protein-uria states (a form of over flow protein uria) or released during tubular cell apoptosis. Fetuin-A was detected in apoptotic vascular smooth muscle cells[25] and apoptotic cells have been demonstrated in tubular cells in cisplatin-or ischemia and reperfusion (I/R)- induced acute kidney injury (AKI) in animals.[24,26]

Another study verified that fetuin-A increased in urinary exosomes in the early phase of AKI in rats after nephrotoxin injection or ischemia and reperfusion and also increased in intensive care unit (ICU) patients with AKI.[27,9]

The results presented in this study are reasonably consistent with those indicated previously, in which the urinary samples of the patients with chronic kidney disease showed high significant increase in mean levels of fetuin- A and α_1-microglobulin as compared with the urinary sample of the healthy control subjects. Therefore urinary exosomes may provide an avenue for the validation of biomarkers for early diagnosis, classification and monitoring treatment of kidney diseases.

Uric acid is normally excreted through the kidney but circulating levels increase during renal impairment in CKD and this may predict a greater risk of end-stage renal disease.

Moreover, animal model studies showed that hyperuricaemia activates the renin-angiotensin system, induces oxidative stress and reduces renal function.[16]

1.1.3 CONCLUSION

Urinary exosomal Fetuin-A represent a new source of non-invasive urinary biomarkers that can overcome much of the interference from other abundant urinary proteins (albumin, globulin and Tamm–Horsfall protein, etc.).

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