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Evaluation of serum copeptin and heart-fatty acid binding protein as predictor biomarkers for chronic diabetic kidney disease in Egyptian Patients

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Abstract

Chronic diabetic kidney disease and diabetic nephropathy are the leading cause of end-stage kidney disease in developed countries. The objective of this study is to evaluate s-copeptin and heart-fatty acid binding protein (H-FABP) as predictor markers of chronic diabetic kidney disease (CDKD) in type 2 Egyptian diabetic patients. Ninety five volunteers were classified as follows: group I comprised 20 normal subjects, group II consisted of 15 hypertensive patients, group III included 30 type 2 diabetic patients (T2D) with normal renal function, and group IV consisted of 15patients with CDKD treated with renin angiotensin aldosterone system inhibitors and group V comprised15 patients with un-treated CDKD. Serum copeptin, H-FABP, diabetic and renal biomarkers were determined. Highly significant increase in s-copeptin level (p<0.001) in hypertensive, treated CDKD and untreated CDKD group compared to control subjects, while a non-significant change were observed in T2DM group. A moderate increased in H-FABP level was recorded in both treated and untreated CDKD group compared to control subjects. Also, s-creatinine significantly increased in both groups of CDKD. On the other hand, e-GFR, showed highly significant decrease (p<0.001) in CDKD groups, compared to other groups. The diagnostic performance study revealed that, s-copeptin and HFABP showed a less diagnostic value in T2D patient group. In hypertensive patient group s-copeptin showed highest diagnostic information, while H-FABP showed a less accuracy. However in CDKD patient groups, s-creatinine and s-copeptin provided the highest diagnostic value followed by H-FABP according to the area under the curve.Hence, s-copeptin is helpful for the identification of chronic diabetic kidney disease (treated and/or untreated) patients with high risk for a decline in renal function.

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Introduction

Diabetes is among the leading causes of kidney failure. The incidence and prevalence of diabetes mellitus (DM) have grown significantly throughout the world. According to WHO, diabetes was the direct cause of 1.6 million deaths worldwide. Chronic diabetic kidney disease and diabetic nephropathy are the leading cause of end-stage kidney disease in developed countries (Azeem *et al.,* 2017). Diabetic kidney disease is a common and serious complication of diabetes associated with adverse outcomes of renal failure. Early and accurate identification of chronic DKD is of critical importance to improve patient outcomes (Temesgen and Zemenu, 2016).

Pathologically, the kidneys undergo several changes, including deposition (in primarily the mesangium) of extracellular matrix, glomerular basement membrane thickening, proliferative changes, and tubular atrophy, ultimately resulting in interstitial fibrosis and glomerulosclerosis (the final common pathway of many kidney diseases)(Kausik and Julia,2018).

The untreated DKD leads to a significant decrease in life expectancy of patients therefore, prevention of this debilitating condition and early diagnosis and treatment is very important issue (Shahbazian and Rezaii, 2013).

The routine classical evaluation of diabetic kidney disease includes the appearance of microalbuminuria, decreased creatinine clearance and increased serum creatinine.Although microalbuminuria and e-GFR have been considered as the gold standard for early diagnosis of kidney disease in clinical practice, 29.1–61.6 % of individuals with T2DM could have renal impairment even before the onset of microalbuminuria (Andersen *et al.*, 2000).

However, clinical trials have demonstrated that this dogma may be incorrect (Mauer *et al.,* 2009). It was found that e-GFR may be declines before the development of proteinuria, demonstrating that there is an earlier phase of kidney damage that could be detected and targeted with interventions. Also, kidney damage can progress even when microalbuminuria has regressed (Steinke and Mauer, 2008). On the other hand, the precision of creatinine-based GFR estimates is limited in hyper-filtration status. These facts make albuminuria and e-GFR less reliable indicators for early-stage DKD (Chih-Hung *et al.,* 2016). Due to the limitations of e-GFR and albuminuria in the early diagnosis of DKD, enormous efforts have been made to investigate and validate alternative biomarkers in recent decades.

Copeptin, a 39-aminoacids glycopeptide is a c-terminal part of pre-provasopressin (pre-proAVP). Pre-proAVP is a precursor protein which consists of a signal peptide, arginine vasopressin (AVP), neurophysin II and copeptin (Jochberger *et al.*, 2006; Szinnai *et al.*, 2007).

AVP binds to V2 receptors in the distal tubules and collecting ducts promoting water absorption and production of cAMP. cAMP plays an important role in the stimulation of cyst growth in the kidney. It is difficult to measure in epidemiological studies because of platelet binding, a very short *ex-vivo* half-life and a laborious assay. Based on these facts, copeptin is suitable for routine measurements as an alternative to AVP (Struck *et al.*, 2005). Since copeptin reflects AVP concentration, measurement of copeptin can provide valuable information about the severity of kidney disease (Meijer *et al.* 2011).

It is known that hypertension can affect renal blood vessels by progression of inflammation. Nelson *et al.* (1995) have shown that the mean blood pressure level at an early stage of diabetes can predict the occurrence of proteinuria after diabetes, believing that hypertension is not only be the result of CKD, but also an initiating factor to promote its occurrence.

Jian, *et al.*(2014) proved that in the population with T2DM, increased systolic BP might contribute to the occurrence and development of chronic DKD.

H-FABP is a cytoplasmic protein with a molecular weight of 15 kDa, which mediates the passage of fatty

acids from the plasma membrane to sites of lipid synthesis. It has been reported that H-FABP is a potent inducer of cardiac myocyte hypertrophy, stimulating an increase in cell surface area, protein synthesis.It is expressed primarily in heart, and to a lesser extent in skeletal muscle, brain and kidneys (Undurti N. Das, 2016).

Jo*et al., 2012* reported that the serum H-FABP is eliminated via renal clearance, the concentrations of H-FABP may be increased in subjects with a decreased renal function.

To delay the onset of chronic kidney disease in diabetic patients, systematic screening and appropriate management are needed. Consequence, development of new assays for diagnostic of chronic diabetic kidney disease has always been the priority in the research field of diabetic complications (Ezz and Azeem, 2016).

In accordance, this study was constructed toevaluates-copeptin and H-FABP as biomarkers for chronic diabetic kidney disease in Patients with type 2 diabetes mellitus and to correlate them with differentrenal markersinEgyptian patients.

Subjects and methods

Subjects

This study included 95 volunteers of both sexes; the mean ages were (50±5.1). They were classified as 20 normal healthy subjects (group I), fifteen hypertensive subjects Group II): they were treated with anti-hypertensive (renin angiotensin aldosterone system inhibitors), and 60 type 2 diabetic patients.

The diabetic patients were subdivided into three groups: (group III) diabetics with normotensive (30 patients), (group IV): fifteen diabetics with chronic diabetic kidney disease (according to criteria of Kidney Disease Improving Global Outcomes (KDIGO) in 2012), they are treated with renin angiotensin aldosterone system inhibitors (CDKD- treated) and group (V): fifteen diabetics with chronic diabetic kidney disease, they are untreated with renin angiotensin aldosterone system inhibitors (CDKD)-Untreated).

All patients were selected from the outpatient clinic of the National Institute of Diabetes and Endocrinology (NIDE) and National Institute of Kidney Diseases & Urology (NIKDU), Cairo, Egypt. Type 2 diabetes mellitus was diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.

The duration of the disease was 6.2±0.5 years in diabetic and hypertensive patients and 4.3±0.21 years in chronic diabetic kidney disease (CDKD) patients. Systolic and diastolic blood pressures were 119.4±2.7 and 75.5±6.2 mmHg for normal subjects, 131.6±7.8 and 85.8±5.2 mmHg for hypertensive patients. 121.6±3.8 and 77.8±5.2 for diabetic normotensive patients, and 158.5±12.3 and 95± 3.7 mmHg for CDKD patients.

The participants in the diabetic groups were uncontrolled type 2 diabetic patients treated with oral hypoglycemic agent with a dose adjusted according to the state of each patient. Patients with any history of thyroid dysfunction, respiratory disorder, liver disease, chronic inflammation, and clinically significant infectious diseases were excluded from the study.

The purpose and nature of the study were explained to all subjects and written voluntary consents were obtained before their participation. Approval was taken from the research committee of General Organization of Teaching Hospitals and Institutions.

Methods

Blood samples were collected into plain vacutainer tubes, after an overnight fasting. Blood was then centrifuged at 3000 rpm for 10 min at 4 °C. Serum samples were rapidly separated and stored at –80 ° C until the measurements. Another part of blood was taken on EDTA for determination of HbA1c levels. For glucose estimation, potassium fluoride was added to tubes. Hemolysed samples were excluded. Fresh

morning urine samples were obtained for measurement of Cr concentration and e-GFR.

Commercial kits were purchased from BioMed. (Egy Chem, Egypt).Plasma glucose concentrations were assayed at once by glucose oxidase method according to Trinder (1969). HbA1c % was measured according to the method of Grey *et al.*[21] using an immunoturbidimetric assay on Dimension RxL Max (Dade Behring).

Lipid parameters, including TC, TAG, HDL-C, LDL-C and kidney functions, including serum and urinary creatinine and protein in urine were detected by biochemical auto-analyzer.

Estimated Glomerular filtration rate (e-GFR) was calculated using the Cockcroft and Gault formula according to Burkhardt *et al.* (2002) and was normalized per 1.73 m2 of body surface area.

H-FABP was quantitatively assayed by ELISA technique. The kit was supplied from Sino Gene Clon Biotech Co., LTD. Serum copeptin was quantitatively assayed by ELISA technique.The kit was supplied from Hangzhou Eastbiopharm Co., LTD.

Statistical analysis

Statistical analysis was performed using the statistical package for the social science (SPSS) for windows (version 22.0, Chicago, IL, USA). Data are presented as means ±SE. The data were analyzed by one-way analysis of variance (ANOVA). A *P*-value less than 0.05 was considered statistically significant.

Pearson's correlation coefficient analysis was used and receiver operating characteristic (ROC) curve was performed to define the sensitivity and specificity of s-copeptin and H-FABP as predictive biomarkers.

Results

The results of the current study were summarized in Table (1).

Data presented in Table 1 revealed that a highly significant elevation of FBS, and HbA1c ($p<0.001$) in diabetic patients group, CDKD patients group, treated with renin angiotensin aldosterone system inhibitors, and CDKD untreated by (95.1 and 47.4%, respectively),(137.1 and 140.2%, respectively) and (153.7and 145.5%, respectively) respectively when compared to control group.

Table 1. Anthropometric data and some biochemical parameters for studied patients groups.

Groups / Parameters	Group I	Group II	Group III	Group IV	Group V
Number	20	15	30	15	15
Age(year)	49±5.2	52±3.1	53±2.4	51±3.5	52±2.3
Sex	M 12/ F 8	M 9/ F 6	M 17/ F 13	M 7/ F 8	M 9/ F 6
Duration	------	6.1±0.15	6.2±0.5	4.3±0.26	4.0±0.21
SBP(mmHg)	119.4±2.7	131.6±7.8	121.6±3.8	158.5±12.3	156.5±11.3
DBP(mmHg)	75.5±6.2	85.8±5.2	77.8±5.2	94± 3.3	95± 3.6
FBS (mg/dl)	91.58±3.2	98.47 ±4.1	178.66±11.9**	217.11±13.9**	232.36±18.3**
HbA1c %	4.85±0.1	4.61±0.4	7.15±0.2**	11.65±0.39**	11.91±0.25**
s-Copeptin (pmol/L)	2.58± 0.13	8.97±0.30**	2.99±0.28	12.02±0.62**	12.9.38±1.12**
H-FABP (ng/ml)	135.79±3.92	145.36±5.60	129.89±6.50	167.2±14.71*	171.28±24.12*
Ur-Alb (mg/l)	15.11±.78	19.23±.68	17.10±0.74	180.02±23.13**	175.8467±27.67**
U-Cr. (g/l)	118.11±3.92	102.53±3.22	116.33±1.62	84.46±2.65*	77.73±1.74**
A/ C Ratio (mg/ g)	10.40± 0.03	12.5± 0.22	9.99± 0.06*	240.20±2.5**	254.33± 7.6**
Serum Cr. (mg/dl)	0.78± 0.03	1.1±0.31	0.77± 0.03	1.98± 0.05**	2.19±0.06**
e-GFR(ml/min)	107.18±3.7	100.23±1.3	108.52±3.0	77.76±2.4**	44.54±2.2**
TAG(mg/dl)	112.8±8.6	156.22±15*	131.1±11.39	180.11±80**	187.07±6.58**
TC (mg/dl)	161.8±6.41	201.72±3.5*	183.7±8.58	240.15±7.30*	249.5±7.2*
LDL-c (mg/dl)	102.5±4.37	125.27±3.73	117.4 ±6.2	155.27±6.7*	149.37±8.23 *
HDL-c (mg/dl)	53.27±2.33	45.37±1.54	46.35±2.54	40.31±3.25	42.01±2.13

Data are expressed as mean ± SE..

FBS, fasting blood sugar; HbA1c, hemoglobin A1c; H-FABP, heart fatty acid binding protein; Alb, albumin; Cr, creatinine; Alb/Cr. ratio, albumin creatinine ratio; e-GFR,estimated glomerular filtration rate; *:Significant from control ($p<0.05$), **: High significant from control ($p<0.001$).

A highly significant decrease (p<0.001) in e-GFR and u- creatinine by (-27.4% and-28.6%, respectively) and (-58.5% and -34.3%, respectively) in treated CDKD and untreated CDKD patient groups respectively.

In contrast, s-creatinine, u- albumin and A/C ratio showed highly significant increase (p<0.001) (157.1 %, 1092.0 % and 2209.6%, respectively) in treated CDKD and (184.4%, 1064.2% and 2342.3 %, respectively) in untreated CDKD patient groups when compared to control group. Also, a significant change was observed in lipid profile, in all patients groups as shown in Table 1. s-Copeptin and H-FABP showed a non-significant changed in DM group, a significant increase was recorded in s-copeptin in hypertensive group by (256%), this increment was augmented in both treated CDKD and untreated CDKD patient groups by (365% and 400%, respectively) when compared to control group.

Table 2. Pearson's correlation coefficients (r) between serum copeptin and some serum biochemical parameters in the patient groups.

s-copeptin	AER	s-creatinin	s-HFABP	e-GFR
Hypertensive group:Pearson's correlation (r)	-------	0.24	0.45	----
Treated CDKD:Pearson's correlation (r)	0.52	0.69	0.62	0.6
Untreated CDKD:Pearson's correlation (r)	0.60	0.56	0.65	0.56

In the meantime, H-FABP showed a non-significant changed in hypertensive group (7%), and significant changed in treated CDKD and untreated CDKD patient groups by (23.2 and 26.1%, respectively) when compared to control group.

Correlation study

There was a moderate significant positive correlation between copeptin and both HFABP and s- creatinine (r: 0.45 and 0.24, respectively) in hypertensive patients, Additionally in treated CDKD and untreated CDKD patient groups highly significant positive correlation was observed between copeptin and AER, s-creatinine and s-HFABP with (r: 0.52, 0.69 and 0.62 respectively) and (r 0.60, 0.56 and 0.65 respectively) (Table 2).

ROC analysis of some biomarkers

ROC analyses were performed, it showed that s-copeptin and HFABPIn diabetic group has a less accuracy and did not provide diagnostic information (AUC: 0.31and 0.45, respectively) (Fig. 1).While in hypertensive groups-copeptin showed highest diagnostic information (AUC: 0.751). In contrast, HFABPshowed a less accuracy performance AUC: 0.20. Meanwhile in treated and untreated CDKD, s-copeptin and s-creatinine showed highest diagnostic information (AUC: 0.904 and 0.901) and (0.97 and 0.96) respectively. While HFABP showed a moderate value (AUC: 0.75 and 0.72), respectively.

By collecting all patients of chronic diabetic kidney disease in one group, It was noted that, s-creatinine and s-copeptinprovided the highest diagnosticinformation (AUC: 1.0 and 0.99, respectively) followed by HFABP (AUC: 0.81).

Discussion

DN is a common and serious complication of diabetes associated with adverse outcomes of renal failure. Early and accurate identification of DN is of critical importance to improve patient outcomes. Changes in the renal tubules, which may be termed diabetic tubulopathy, are increasingly implicated in the development of progressive chronic kidney disease. It has been reported that, in addition to the glomeruli, the renal tubules are heavily involved in the pathogenesis of DN (Temesgen and Zemenu, 2016).

Renal dysfunction is reported to correlate with the degree of tubulointerstitial damage. Although albuminuria per se reflects glomerular damage and

subsequently induces renal tubulointerstitial damage, other factors and mechanisms, independent of albuminuria, must be involved in the development of tubulointerstitial damage under diabetic conditions (Vallon, 2011).

To improve prognosis, it is important to predict the incidence of renal disease in type 2 diabetic patients before the progression of advanced nephropathy. Therefore, current study was constructed to investigate the predictive values of both s-copeptin and H-FABP as early markers for renal tubulointerstitial damage in diabetic patients.

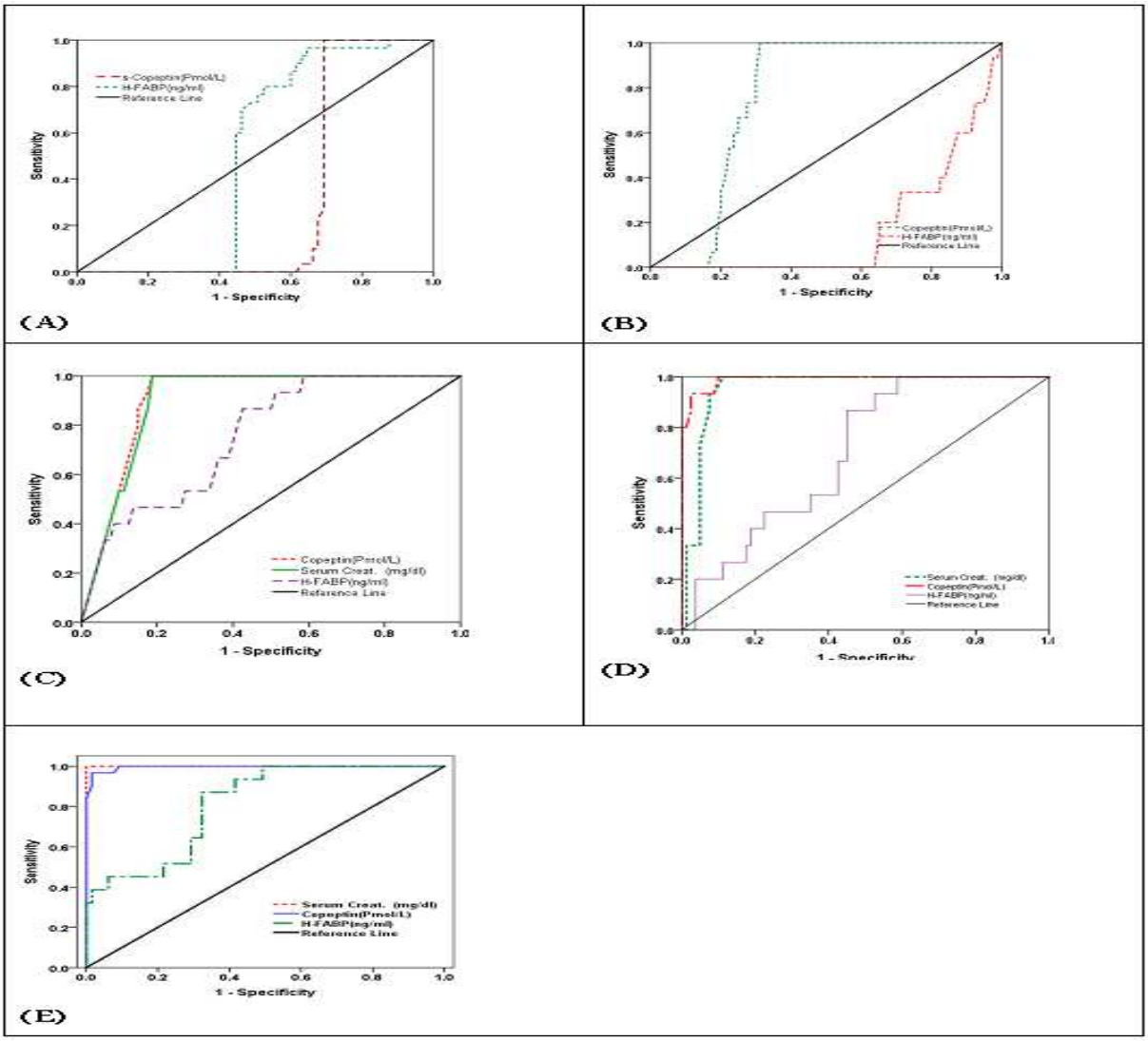

Fig. 1. Receiver operating characteristic curves displaying the accuracy of s-copeptin, HFABP and s-creatinine, (A):diabetic group, (B): hypertensive group, (C): treated CDKD, (D): untreated CDKD and (E): collective group.

The patients in this study were chosen as hypertensive patients where in the population with T2DM, increased systolic BP might contribute to the occurrence and development of chronic DKD (The underlying mechanism has not yet clear). Also increased blood pressure level, is a more critical contributing factor causing myocardial damage and kidney disease (Jian, *et al.*, 2014). The results of this study showed that, therenal biomarkers include s-Cr, u-Cr and e-GFR were not significantly changed in DM and hypertensive patient groups compared to control subjects. On the other hand, s-Crand A/C ratio were highly significantly increased inpatients with CDKD treated with renin angiotensin aldosterone system inhibitorsand in un-treated CDKD patient groups compared to normal control subjects. In contrast, e-

GFR and u-Cr were significantly decreased in patients with CDKD treated with renin angiotensin aldosterone system inhibitorsand in un-treated CDKD patient groups A/C ratio showed a non- significant change in diabetic and hypertensive patient groups.

Kramer *et al.*(2007) clarified that the routine classical evaluation of diabetic kidney disease includes the appearance of microalbuminuria, decreased creatinine clearance and increased serum creatinine.

It has been reported that a decline in the renal function of patients with diabetes was not always accompanied by albuminuria and reduced in e-GFR. About 20%-30% of patients with type 2 diabetes, accompanied by renal insufficiency, showed normoalbuminuria. Several tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and a rise in serum creatinine (Uslu *et al.,* 2005).

At present, urinary albumin-to-creatinine ratio and estimated glomerular filtration rateis the standard method for assessing glomerular damage and renal function changes in clinical practice. However, clinical trials have demonstrated that this dogma may be incorrect (Mauer *et al.,* 2009).

Results of the current study showed that TAG, TC and LDL-c were significant elevated in the hypertensive group, and highly significant increase in treated and untreated CDKD patient groups, while there was non-significant change in the diabetic group when compared to control group.

These results are in the same line with the results of Zhang *et al.*(2015); who reported that, high glucose enhanced lipid accumulation in podocytes. These changes may contribute to kidney disease progression in conjunction with other risk factors. Lipid accumulation in diabetic kidney cells may originate from an imbalance between lipid influx and efflux, including increased lipid uptake and biosynthesis or decreased lipidefflux from cells. Dyslipidemia is an independent risk factor for the development and progression of chronic kidney disease (Kuwabara *et al.*, 2014).

Results obtained from the present study revealed that treated and untreated patient groups of CDKD exhibited highly significant increases in serum copeptin, also significant increase in hypertensive patient group and non-significant change in T2DM when compared to control group.

Sofia *et al.* (2019), in their study concluded that, the key finding is that increased levels of copeptin independently predict decline in e-GFR, and suggest that copeptin can be used to identify individuals at higher risk for development of CKD.

The relation between s- copeptin and development of chronic diabetic kidney disease could be explained in terms of the following:first, as copeptin is cleared by kidney excretion,copeptin levels would tend to increase as kidney function decreases. Second, in patients with lower kidney function, more copeptin is released, because the AVP system is activated due impaired urine concentrating capacity to maintain water homeostasis (Zittema *et al.* 2012). Indeed, longitudinal studies in humans have shown that plasma copeptin levels increase before e-GFR decreases (Ponte *et al.*, 2015).

In most studies copeptin was positively associated with urinary albumin/protein excretion. Population-based studies have shown copeptin to be strongly associated with microalbuminuria (Meijer *et al.*, 2010).

In the present study, a significant positive correlation between copeptin and s- creatinine in hypertensive patients was observed as well asin treated CDKD and untreated CDKD patient groups. In opposite,a negative correlation was recorded between s-copeptein and e-GFR in treated CDKD and untreated CDKD patient groups.

These results are in the same line with the result obtained by Baris Afsar, (2017); who clarified the relationship between copeptin, albuminuria and GFR

in their study, they concluded the following issues: i) Copeptin and GFR is usually negatively correlated ii) Copeptin and albuminuria/proteinuria is positively correlated iii) Copeptin and elevated BP were usually associated with each other Many studies have shown that increased copeptin concentrations are linked to renal insufficiency and copeptin is negatively associated with estimated glomerular filtration agonist was shown to induce glomerular hyper-filtration and to increase UAE in normal rats.

Kimura *et al.*(1999) first reported that H-FABP is secreted in human glomeruli and is localized largely along the capillary wall. To date, most studies on H-FABP have focused on the heart, while the role of H-FABP in the kidney has remained largely elusive.

In the current study, serum H-FABP levels were non-significant changed in DM and hypertensive group, and significant changed in treated CDKD and untreated CDKD patient groups with positive correlation between s-copeptin and H-FABP when compared to control group.

The current results were consistent with Huimei *et al.(* 2012) who clarified that increased levels of H-FABP were correlated with the progression of proteinuria in patients with diabetic nephropathy and obesity- associated glomerulopathy.

While the present results were in contrast with the study of Karbek *et al.* (2011), where they observed that a significant alteration in serum H-FABP levels was detected in patients with pre-diabetes. H-FABP levels were increased in patients with impaired fasting glucose and impaired glucose tolerance.

Huimei *et al.* (2012) noted that, increased expression of H-FABP was directly related with proteinuria level in humans with obesity- associated glomerulopathy.

It has been widely reported that lipid accumulation is related to renal damage (Wang *et al.,* 2005). As lipid-binding proteins, FABPs have proposed roles in fatty acid metabolism and been proven to be involved in the pathological events of the kidney (Zuo *et al.,* 2011).

Also, Huimei *et al.* (2012), concluded that podocyte lesions resulting from the increment of H-FABP expression, where it playing a protective effect on podocyte by attraction of FFA.

The diagnostic performance of different biomarkers for DM, hypertensive and chronic diabetic kidney disease patients groups revealed the following, s-copeptin and HFABP showed less accuracy parameters and did not provide diagnostic information in DM patient group.

In hypertensive patient group s-copeptin showed highest diagnostic information, while H-FABP showed a less accuracy.

However in CDKD, s-creatinine and s-copeptin provided the highest diagnostic value followed by H-FABP according to the area under the curve.

Hence the current study showed that s- copeptin is helpful for the identification of chronic diabetic kidney disease (treated and/or untreated) patients with high risk for a decline in renal function. Thus, s-copeptin may be a useful tubular biomarker for essential hypertension and chronic kidney disease

Conclusion

In CDKD patients, s-copeptin was significantly increased and it significantly positive correlate with s-creatinine. In opposite, a negative correlation was recorded between s- copeptein and e-GFR in the same patients groups. s-copeptin and HFABP showed a less accuracy parameters and did not provide diagnostic information in DM patient group. However in CDKD, s-creatinine and s-copeptin provided the highest diagnostic value followed by H-FABP. Hence, s-copeptin is considered as new tubular biomarker represents kidney state of diabetic patients.

It could be used as a more sensitive marker in predicting progression of CKD in Egyptian Type 2diabetes mellitus patients.

Conflict of interest

Authors have no conflict of interest.

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