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Serum Beta-Trace Protein and Cystatin C as Biomarkers for Renal Dysfunction in Patients with Chronic Kidney Disease

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Abstract

Chronic kidney disease (CKD) is recognized as a major public health threat worldwide with consequences of kidney failure. Around 10% of the global adult population has CKD. Therefore, the current study aims to evaluate the diagnostic role of cystatin C (Cys-C) and beta-trace protein (BTP) as biomarkers for the detection of renal impairment in patients with CKD. The study included 89 individuals classified to healthy volunteers (n=30), patients with renal impairment (pre-hemodialysis) (n=29), and patients undergoing haemodialysis (n=30). The serum levels of Cys-C and BTP were estimated by enzyme-linked immunosorbent assay (ELISA). Also, receiver operating characteristics (ROC) curves and area under the curve (AUC) were calculated. Cys-C and BTP levels were gradually increased in patients with renal impairment followed by patients undergoing hemodialysis in comparison with healthy volunteers. Also, there are significant correlations between the two markers with urea, creatinine, and glomerular filtration rate. ROC curve analyses data revealed that BTP and creatinine showed better diagnostic performance (AUC=1, Sensitivity: 100%, Specificity: 100%, and accuracy: 100%) compared to Cys-C (AUC=0.996, Sensitivity: 96.61%, Specificity: 96.67%, and accuracy: 96.63%). Taken together, these results recommended that BTP and Cys-C are potential markers than creatinine for the early detection of renal impairment in patients with CKD.

Keywords: Beta-trace protein; Cystatin C; Creatinine; Chronic kidney disease

Introduction

Chronic Kidney Disease (CKD) is a broad term used to describe any abnormality of kidney structure or function. It is associated with an increased risk of end-stage renal failure. CKD is a decreased kidney function and characterized by a glomerular filtration rate (GFR) less than 60 mL/min/1.73 m^2 [1].

Serum creatinine is a basic marker of GFR in spite of its numerous well-known restrictions. The serum concentration of creatinine is reliant on various non-renal factors such as gender and muscle mass [2]. Although, it was used for many decades to assess the kidney function, however, it noticed that this marker isn't perfect and accurate [3]. Consequently, the requirement for new biomarkers for monitoring kidney function in patients with CKD was demanded.

Cystatin-C (Cys-C) is a 13-kDa non-glycosylated protein. It synthesized and secreted by several human cells not including renal tubules [4]. Due to its independent on sex, diet, and muscle mass, it considers better than creatinine clearance in the diagnosis of renal function impairment [5]. Also, Cys-C can be used as an alternative marker to creatinine [6,7].

Beta-trace protein (BTP) is a 23–29 kDa protein isolated from cerebrospinal fluid as well as it located with low concentrations in serum and urine. It is stable at urine pH and totally excreted via kidneys [8]. Also, numerous studies revealed that BTP is a talented marker of renal impairment [9–11].

Early detection of CKD may provide an opportunity to avoid progression of associated risks. Therefore, the aim of the current study was to examine the clinical efficacy of serum levels of Cys-C and BTP for the early detection of renal dysfunction in Egyptian patients with CKD.

Materials and Methods

Patients groups

The patient's groups participate in the present study were selected from Menoufia University Hospital, Egypt. A written consent was taken before their participation in the study. There are two groups involved in the study beside the control group. Group 1: patients with renal impairment (pre-hemodialysis) and included 29 individuals (15 male+14 female) with age range 27–85 y. Group 2: patients undergoing hemodialysis (3 times/week) and included 30 individuals (15 female+15 male) with ages range 21–72 y. In addition, healthy control group included 30 individuals (21 male+9 female) with age range 18–70 y.

Samples

Blood samples (8 ml) of each individual were collected under complete aseptic condition and allowed to clot at 37°C. Samples were centrifuged at 4000 rpm for 10 min at 4°C. The clear supernatants were collected, aliquoted, and stored at (–20°C) till the time of investigation.

Laboratory measurements

Estimation of creatinine level: The serum creatinine level was assessed according to Jaffe assay [12] using human creatinine kit (Roche-Germany). Briefly, creatinine in samples reacted with picric acid in an alkaline solution and the intensity of the developed coloured complex was measured.

Assessment of urea level: Urea level in serum samples was measured by enzymatic method using urea kit (BioMerieux-France). In brief,

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urea in samples was hydrolysed by urease to form ammonium and carbonate then ammonium reacts with 2-oxoglutarate. In the reaction, NADH was oxidized to NAD+ and the decreasing rate in NADH concentration was directly proportional to the urea concentration in the sample [13].

GFR valuation

The estimated GFR (eGFR) is determined by combining the serum creatinine level with other parameters such as age and gender using the Chronic Kidney Disease Epidemiology Collaboration (CKD–EPI) equation [14].

Measurement of Cys-C and BTP concentration

Cys-C and BTP concentrations in samples were measured using ELISA kits (Zhejiang Kono Biotech co., Ltd, Zhejiang, China) according to the manufacturer's instructions. Briefly, microtiter plate was coated with human Cys-C and BTP antibody followed by addition of serum sample (10 µl) to each well then 50 µl of horseradish peroxidase (HRP) conjugate was added. The plate was sealed, gently shacked, and incubated for 1 h at 37°C. After aspiration and washing, 50 µl of chromogen A and B were added to each well then, the plate was gently shacked and incubated for 10 min at 37°C. Lastly, stop solution (50 µl) was added into each well to stop the reaction. The absorbance of the developed colour was immediately measured at 450 nm. The concentrations of Cys-C and BTP in samples were determined using standard curve.

Statistical analysis

The statistical analysis was performed by SPSS software (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± SD. Differences of continuous variables between the different groups' data were assessed by independent samples t-test. Pearson correlation was used to assess the correlation between parameters of interest. The predicted probability of predicting renal impairment was used to construct ROC curve. The diagnostic efficacy of each panel was assessed by AUC. The optimal cut-off values for diagnosis were selected using Youden's index, which were maximal values at the sum of the sensitivity and specificity.

Results

Demographic parameters of the studied groups

The demographic data of healthy control group and patients with renal impairment and undergoing hemodialysis groups shown in Table 1.

Serum levels of urea, creatinine and eGFR

Table 2 showed that patients with kidney impairment had three-fold elevations in the levels of serum urea and creatinine with recorded main values 91 and 2.47 mg/dl compared to 30 and 0.79 mg/dl, respectively in healthy control group, however, eGRE level declined to 38 ml/min in kidney impairment group, compared to 134.6 ml/min in healthy control group.

Conversely, in comparison with the healthy control group, patients undergoing hemodialysis had the maximum elevated levels of serum urea and creatinine and the noticed values were 125 and 9.96 mg/dl, respectively, while, eGFR level declined to 9.8 ml/min. The levels of serum urea, creatinine, and eGFR in patients undergoing hemodialysis were significantly (P<0.001) higher than those of healthy controls and kidney impairment.

Variables	Groups (Mean ± SD)			*P*- value
	Control	Renal impairment (Pre-hemodialysis)	Undergoing Hemodialysis	
N	30	29	30	--
Gender (M/F)	21/9	15/14	15/15	--
Age (Year)	47 ± 16	54 ± 13	53 ± 14	P_1= 0.071 P_2 = 0.097 P_3 = 0.06
Height (M)	173 ± 8	172 ± 8	169 ± 9	P_1 = 0.649 P_2 = 0.073 P_3 = 0.166
Weight (Kg)	77 ± 11	80 ± 7	74 ± 17	P_1 = 0.381 P_2 = 0.318 P_3 = 0.083
BMI (Kg/m²)	26.2 ± 4.7	27.1 ± 3.1	25.9 ± 5.8	P_1 = 0.374 P_2 = 0.854 P_3 = 0.331
Note: BMI = Body Mass Index P_1: Statistical difference between control and renal impairment (pre-hemodialysis) group. P_2: Statistical difference between control and undergoing hemodialysis group. P_3: Statistical difference between renal impairment (pre-hemodialysis) group and undergoing hemodialysis group.				

Table 1: Demographic data of the studied groups.

Variables	Groups (Mean ± SD)			*P*- value
	Control	Renal impairment (Pre-dialysis)	Undergoing hemodialysis	
Urea (mg/dl)	30 ± 7	91 ± 19	125 ±1.40	0.001
Creatinine (mg/dl)	0.79 ± 0.19	2.47 ± 0.63	9.96 ± 3.2	
eGFR (ml/min)	134.6 ± 44.8	38 ± 12.7	9.8 ± 5.5	
Note: eGFR: estimated glomerular filtration rate				

Table 2: Serum levels of urea, creatinine and estimated GFR.

Variables	Groups (Mean ± SD)			*P*- value
	Control	Renal impairment (Pre-dialysis)	Undergoing hemodialysis	
BTP (pg/ml)	480 ± 40	752 ± 140	2903 ± 3173	0.001
Cys-C (ng/ml)	72 ±14	312 ± 187	1486 ± 1066	
Note: Cys-C = Cystatin C; BTP = beta trace protein				

Table 3: Serum concentrations of BTP and Cys-C.

Variables	BTP		Cys-C	
	Renal impairment (Pre-dialysis)	Renal Impairment & Hemodialysis	Renal impairment (Pre-dialysis)	Renal Impairment & Hemodialysis
Urea	0.801**	0.549**	0.706**	0.697**
Creatinine	0.973**	0.772**	0.908**	0.906**
eGFR	- 0.8**	- 0.364**	- 0.655**	- 0.52**
Note: *r*: Pearson correlation; + *r*: Positive correlation (direct); - *r*: Negative correlation (inverse); **: *P*<0.01				

Table 4: The correlations of BTP and Cys-C levels with urea, creatinine, and eGFR.

Serum concentrations of BTP and Cys-C

As shown in Table 3, patients with kidney impairment had higher levels of serum BTP and Cys-C (752 pg/ml and 312 ng/ml, respectively) compared to healthy control group (480 pg/ml and 72 ng/ml, respectively). On the other hand, patients undergoing hemodialysis exhibited the maximum elevated levels of serum BTP and Cys-C (2903 pg/ml and 1486 ng/ml), respectively in comparison with control group. The statistical difference (P=0.001) was detected between the two groups.

Markers	Cut-off value	AUC	Sig.	95% CI		Sensitivity %	Specificity %	Accuracy %
				Lower bound	Upper bound			
Creatinine	1.35	1	0.001	1	1	100	100	100
Cys-C	102.5	0.996		0.989	1.003	96.61	96.67	96.63
BTP	555	1		1	1	100	100	100
Note: AUC: Area Under Curve; Sig: Significance; CI: Confidence Interval; Cys-C: Cystatin C; BTP: Beta Trace Protein								

Table 5: ROC curve analyses data for creatinine, Cys-C, and BTP.

The correlations of BTP and Cys-C levels with urea, creatinine and eGFR

The correlations of urea, creatinine and eGFR with the serum levels of BTP and Cys-C in patients with renal impairment and/or undergoing hemodialysis were demonstrated in Table 4. In renal impairment patients, there were positive correlations between BTP and Cys-C with urea (r=0.801 & 0.706, respectively) and creatinine (r=0.973 & 0.908, respectively), while, GFR showed a negative correlation with BTP and Cys-C (r=- 0.8 & - 0.655, respectively). Conversely, the correlations of BTP and Cys-C in patients with renal impairment and undergoing hemodialysis showed positive correlations with urea (r=0.549 & 0.697, respectively), creatinine (r=0.772 & 0.906, respectively), however, there is a negative correlation with GFR (r=- 0.364 & - 0.52, respectively).

Receiver operating characteristics (ROC) curve analyses

As revealed in Table 5, ROC curve analysis data for creatinine revealed that at cut-off value 1.35 mg/dl, it exhibited area under curve (AUC) value equals 1 and percent values (100%) for sensitivity, specificity, and accuracy. Concerning to Cys-C, at cut-off value 102.5 ng/ml, it showed AUC=0.996 and the values of 96.61%, 96.67%, and 96.63% were noticed for sensitivity, specificity, and accuracy, respectively. Whereas, BTP at cut-off value 555 pg/ml, it presented AUC=1 and the percent values (100%) were observed for sensitivity, specificity, and accuracy.

Discussion

Kidneys are essential organs in the bodies due to their vital functions to remove waste products, excess water, and regulate the acidity balance of the blood. CKD is the loss of the kidneys' capabilities to achieve these essential functions. Also, it is defined as structural or functional kidney damage with the detected value of GFR <60 mL/min/1.73m^2 [15].

CKD is a huge public health problem and represents about 10% of the worldwide population [16]. Also, it is the 12th most common cause of death and the mortality has augmented by 31.7% over the last 10 years, making it one of the swiftest growing major causes of death [17]. Early detection of CKD will help in the reduction of the progression of associated risks. Hence, the current study aims to estimate the clinical efficacy of the serum levels of Cys-C and BTP for the early detection of renal dysfunction in patients with CKD. The details of the patients are cited in Table 1.

Urea and creatinine levels are important biomarkers as they have an important diagnostic role in kidney failure [18–20]. The results in Table 2 showed that serum urea and creatinine in patients with kidney impairment had elevated three times more than the detected level in healthy individuals. Whereas, patients undergoing hemodialysis had the maximum elevated levels of serum urea and creatinine. Also, eGFR level was declined in CKD patients and the individuals undergoing hemodialysis recorded the lowest level compared to healthy individuals (Table 2). These findings are in agreement with the previous studies proven that the increased plasma creatinine has a renal cause and consider a result of reduced GFR. Also, reduced GFR is related with increased plasma urea concentration [21]. Additionally, GFR is a good measurement of kidney function and well correlated with renal function disorder [22–24].

As shown in Table 3, patients with kidney impairment possess higher levels of serum BTP and Cys-C compared to healthy control group. Conversely, patients undergoing hemodialysis exhibited the maximum elevated levels. These findings are agreed with that reported serum levels of beta-trace protein have been increased in patients with various renal diseases.

Patients with renal impairment or/and undergoing hemodialysis, showed a positive correlation with urea, creatinine, however, there is a negative correlation with GFR (Table 4). These findings were in consistent with previously mentioned that elevated BTP concentrations in CKD patients significantly correlated with the concentrations of creatinine and cystatin C [25]. Also, similar correlation was reported between BTP and each of creatinine and cystatin C [26].

Our results showed that ROC curve analysis data revealed that creatinine and BTP exhibited higher sensitivity, specificity, and accuracy values (100%) than Cys-C (96.61%, 96.67%, and 96.63%), respectively as shown in Table 5. These conclusions were in consistent with the previously stated that cystatin-C and BTP are better than creatinine and proposed to be highly helpful in the case of GFR decreasing [27–30]. Also, BTP and cystatin C are independent of age and gender [31,32] and appropriate to detect early renal function impairment [33]. Moreover, cystatin-C could function as a potential marker in detecting early renal dysfunction [34].

In summary, our results proven that BTP and Cys-C are proper than creatinine and may be potential markers for the detection of renal dysfunction in patients with CKD.

Conclusion

Cys-C and BTP levels showed an elevation in the sera of patients with renal impairment and those undergoing hemodialysis. Cys-C and BTP were significantly correlated with urea, creatinine, and eGFR. Serum BTP might be a potential marker than Cys-C due to its high sensitivity and specificity. BTP might be a promising diagnostic biomarker for patients with CKD with a recommendation that future studies on large scale are required to confirm the obtained results.

Disclosure of Conflict of Interest

None.

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