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Beta trace protein level as a better diagnostic marker of renal impairment in patients with chronic kidney disease, diabetes mellitus, and renal transplants

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Abstract

Chronic kidney disease (CKD) is a general term for heterogeneous disorders affecting the structure and function of the kidney. Beta trace protein (BTP), also known as prostaglandin D2 synthase, is a low-molecular weight protein which belongs to the lipocalin protein family. It was found to be increased in the serum of patients with renal diseases. **Aim of study:** To compare the clinical usefulness of serum levels of BTP for the detection of renal dysfunction in patients with chronic kidney disease (CKD) and make a comparison with levels of other renal markers creatinine, cystatin C.

Methods: The study included 150 patients divided into three groups with a wide range of renal dysfunction that encompassed CKD stages from (I-IV).

Results: The obtained data showed that BTP was highly correlated (pearson test) with measured GFR (mGFR) (r = 0.86) in logarithmic linear model, and correlated with creatinine (r = 0.558), cystatin C (r = 0.583).

Conclusion: BTP may be a useful and reliable serum marker for identifying the magnitude of renal dysfunction in patients with CKD and may have its place beside serum cystatin C and creatinine as an alternative endogenous GFR marker.

Key Words : Beta Trace Protein, Renal impairment ,CKD,DM,RT,GFR

INTRODUCTION

Chronic kidney disease (CKD) is a general term for heterogeneous disorders affecting the structure and function of the kidney (i.e., persistent urine abnormalities, structural abnormalities or impaired excretory renal function suggestive of a loss of functional nephrons) [1]. The Kidney Disease Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation (NKF) have been defined CKD by either GFR < 60 ml/min/1.73 m² or the presence of kidney damage for more than 90 days duration via clinical assessment (Duration is necessary to distinguish chronic from acute kidney disease) [2,3].

Beta trace protein (BTP) , or prostaglandin D2 synthase (PGDS) is a low molecular weight heterogeneous monomeric glycoprotein with 168 amino acids. Traditionally been used as a marker for cerebrospinal fluid (CSF) leakage because it represents approximately 3% of the proteins in the CSF [4]. BTP acts as a prostaglandin D synthase, which is an enzyme that catalyzes the conversion of prostaglandin H2 (PGH2, a common 50 precursor of various prostanoids) to prostaglandin D2 (PGD2), with a t**1/2** of about 1.2 hours, and almost "completely" it is excreted by the kidneys[5]. In recent years, there have been many more studies describing the similar diagnostic performance of BTP to cys.C as a marker of GFR (compared to the conventional methods, serum BTP have been shown to be more helpful for estimating GFR; Serum BTP measurement can be a reliable tool for detecting kidney function in neonates) [6,7].

Cystatin C is freely filtered by the glomerulus and is largely reabsorbed and catabolized in the proximal tubules, but not secreted, by the renal tubules[8,9]. Cys. C has been proposed by some as a more ideal endogenous biomarker of chronic kidney function. There may a casting doubts on its usefulness as a glomerular filtration endogenous marker[10,11]. Concerning kidney transplantation, cys. C, measured by the "particle-enhanced turbidimetric immunoassay" (PETIA), underestimates GFR in kidney transplant patients [12].

Creatinine generation is direct proportion to muscle mass, which can be estimated from age, gender, race, and body size [13,14,15]. It is an endogenous biomarker with low mol.wt. Make it freely filtered at the glomerulus. It is concentration is inversely related to GFR as a GFR marker, it is convenient and inexpensive to measure so, it is widely used as an endogenous marker of GFR in routine clinical assessment [16]. Moreover, a small (but significant) and variable amount of the creatinine appearing in the urine is derived from "tubular secretion": about, 7 to 10 % is due to tubular secretion [17]. But, this amount is increased in the presence of renal insufficiency. Many preanalytical and analytical limitations, recommended that serum creatinine measurement alone is not used to assess renal function [18].

MATERIALS AND METHODS

Subject samples

The study was performed in a population of 150 patients (61 females and 139 males) with chronic kidney disease. Serum samples were collected from each patient. Serum concentrations of creatinine, cystatin C and were measured on the day of blood collection, and BTP was measured later in serum samples stored at –80 °C. All study participants gave written informed consent.

Methods

BTP was measured by enzyme linked immunosorbent assay (ELISA) Cat. No.: RD191113100R by BioVendor – laboratorni medicina a.s. . BTP was also measured in the sera of 50 healthy volunteers (18 females and 32 males) with mean ± SD age 29.76 ± 13.1 years and the mean concentration (± SD) was 0.46 ± 0.07 mg/L.

Enzyme linked immunosorbent assay (ELISA) with a Cat. No.: EH0110 was also used to measure cystatin C, and creatinine was measured by a kinetic alkaline picrate method [19].

Measured GFR (mGFR), by using ^{99m}Tc-DTPA radio isotopic technique as a standard method.

Statistical analysis

All statistical analysis were carried out by the aid of SPSS software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. USA) and Microsoft Excel (2010, Microsoft Corp. USA). Taking $P < 0.05$ as a significant result. One way ANOVA was employed to evaluate the presence of significant difference of measured parameters among studied groups.

RESULTS

Patient groups general characteristics are summarized in table 1.

Table 2. summarized the mean ± SD of serum concentration of BTP, cystatin C, and creatinine in control and renal disease groups. The mean ± SD of BTP serum concentration in control group was 0.46 ± 0.07 mg/l, regarding cystatin C it was 0.7 ± 0.06

mg/l, while creatinine, it was 0.74 ± 0.11 mg/dl, as shown in figure 1.

BTP had been shown a higher significant concentration in all renal disease group ($P < 0.05$) comparing to control group table (3). Concerning serum concentration of cystatin C which shown also a significant higher concentration in renal transplant and CKD groups but shown no significant difference ($P > 0.05$) in diabetes comparing to control group table (4) as was the case regarding serum creatinine concentration table (5).

Pearson correlation had been shown that there was a significant negative correlation ($P < 0.05$), $r = -0.799$ between serum concentration of BTP and mGFR (using ^{99m}Tc-DTPA as an exogenous standard marker). The analysis had been shown that, the logarithmic linear model of correlation represent the best prediction of mGFR ($r = 0.86$). whereas the linear model of correlation provided only minimally diverging results ($r = 0.799$). At the same time, it shown also a significant negative correlation ($P < 0.05$), $r = -0.591$ whereas serum concentration of creatinine ($P < 0.05$), $r = -0.661$. the characteristics of these correlations are summarized in figure 2.

Table 1. group characteristics (n = 200)

Group	Sample size	Mean(±SD) Age (y)	male(%)	female(%)
Control	50	29.76±13.1	32 (64%)	18 (36%)
CKD	50	33.14±22.7	28 (66%)	22 (44%)
RT	50	45.8±12.8	50 (100%)	0 (0%)
DM	50	46.6±14.8	29 (57%)	21 (43%)

Table 2. mean ± SD of serum concentration of BTP, cystatin C, and creatinine in control and renal disease groups.

Groups	N	Serum BTP		Serum Cystatin C		Serum Creatinine	
		Mean	S.D	Mean	S.D	Mean	S.D
Control	50	0.46	0.07	0.7	0.06	0.74	0.11
CKD	50	0.88	0.26	0.83	0.2	0.81	0.19
RT	49	1.36	0.36	1.39	0.62	1.03	0.15
DM	49	0.69	0.22	0.69	0.11	0.74	0.16
Total	198	0.85	0.41	0.9	0.44	0.83	0.19

Table (3). BTP serum level (mg/l) mean and significant differences among control and renal disease groups

BTP	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Significant P.value	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Control	CKD	- 0.42*	0.05	0.000	- 0.52	- 0.32
		RT	- 0.9*	0.05	0.000	- 0.99	- 0.8
		DM	- 0.23*	0.05	0.000	- 0.33	- 0.13
	CKD	control	0.42*	0.05	0.000	0.32	0.52
		RT	- 0.47*	0.05	0.000	- 0.57	- 0.37
		DM	0.19*	0.05	0.000	0.09	0.29
	RT	control	0.9*	0.05	0.000	0.8	0.99
		CKD	0.47*	0.05	0.000	0.37	0.57
		DM	0.66*	0.05	0.000	0.56	0.76
	DM	control	0.23*	0.05	0.000	0.13	0.33
		CKD	- 0.19*	0.05	0.000	- 0.29	- 0.09
		RT	- 0.66*	0.05	0.000	- 0.76	- 0.56

*. The mean difference is significant at the 0.05 level.

Table (4). Scys.C serum level (mg/l) mean and significant differences among control and renal disease groups

	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Significant P.value	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Control	CKD	- 0.13	0.06	0.051	- 0.26	0.0006
		RT	- 0.69*	0.06	0.000	- 0.82	- 0.56
		DM	0.002	0.06	0.971	- 0.13	0.13
	CKD	control	0.13	0.06	0.051	- 0.0006	0.26
		RT	- 0.56*	0.06	0.000	- 0.69	- 0.43
		DM	0.13*	0.06	0.048	0.0012	0.26
	RT	control	0.69*	0.06	0.000	0.56	0.82
		CKD	0.56*	0.06	0.000	0.43	0.69
		DM	0.69*	0.07	0.000	0.56	0.82
	DM	control	- 0.002	0.06	0.971	- 0.13	0.13
		CKD	- 0.13*	0.06	0.048	- 0.26	- 0.0012
		RT	- 0.69*	0.07	0.000	- 0.82	- 0.56

*. The mean difference is significant at the 0.05 level.

Table (5). Scr level (mg/dl) mean and significant differences among control and renal disease groups

	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Significant P.value	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Control	CKD	- 0.07*	0.03	0.029	- 0.13	- 0.007
		RT	- 0.28*	0.03	0.000	- 0.34	- 0.22
		DM	- 0.0005	0.03	0.988	- 0.06	0.06
	CKD	control	0.069*	0.03	0.029	0.007	0.13
		RT	- 0.21*	0.03	0.000	- 0.27	- 0.15
		DM	0.069*	0.03	0.031	0.006	0.13
	RT	control	0.28*	0.03	0.000	0.22	0.34
		CKD	0.21*	0.03	0.000	0.15	0.27
		DM	0.28*	0.03	0.000	0.22	0.34
	DM	control	0.0005	0.03	0.988	- 0.06	0.06
		CKD	- 0.07*	0.03	0.031	- 0.13	- 0.006
		RT	- 0.28*	0.03	0.000	- 0.34	- 0.22

*. The mean difference is significant at the 0.05 level.

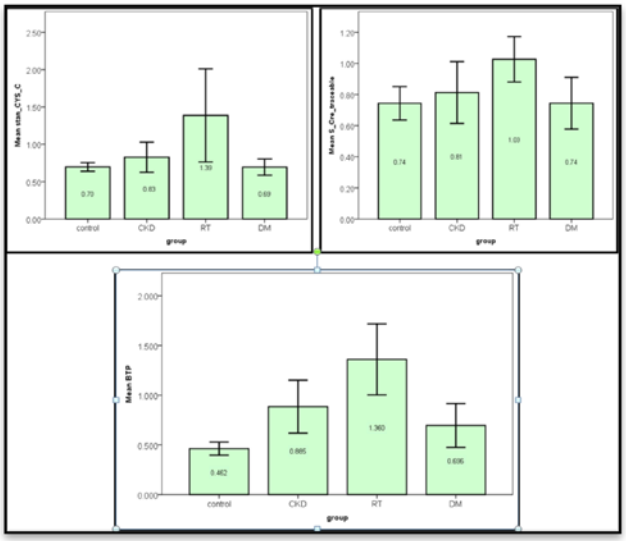

Figure 1. Mean ± SD of serum concentration of BTP, cystatin C, and creatinine in control and patient groups.

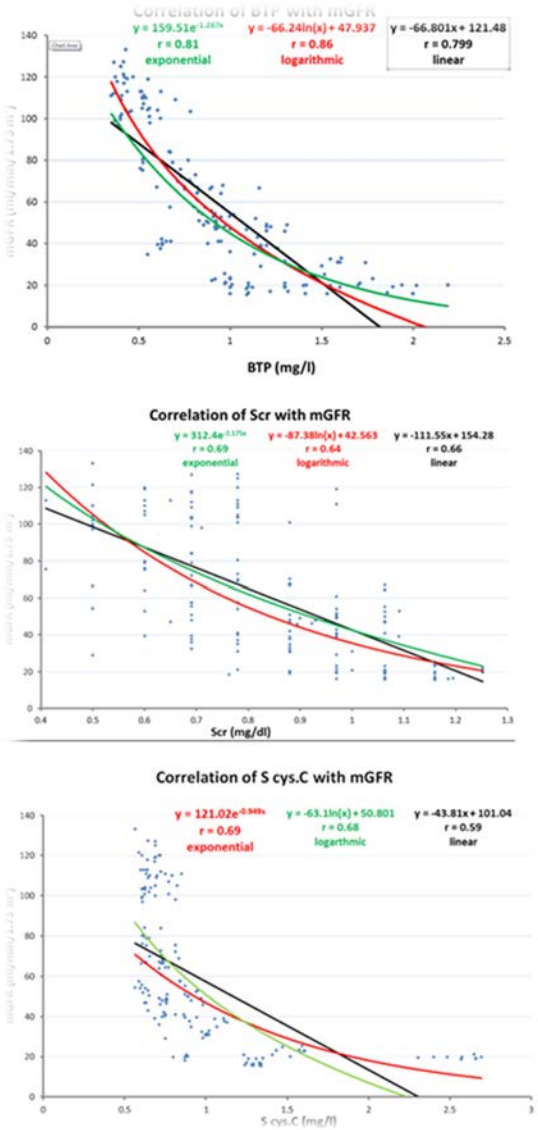

Figure 2. linear model correlation of BTP, cystatin C , and creatinine with mGFR

DISCUSSION

Markedly increased serum levels of BTP had been shown in patients with various renal diseases. The mean ± SD of BTP serum concentration (mg/l) in RT was 1.36 ± 0.36, CKD 0.88 ± 0.26, and in DM was 0.69 ± 0.22. which was in all cases had been shown significant higher level than from its serum concentration in control group 0.46 ± 0.07.

At the same time, BTP of high molecular weight denoted "H-BTP" concentration is elevated in the serum of all renal disease groups in this study comparing to control group more than any other biomarkers had been taken. However, the higher concentration of all markers participate in that are direct proportion and significant correlation with the higher risk of incident End Stage Renal Disease (ESRD) [20], (figure 3) illustrated the results of fold increment over the concentration of the marker for control group.

In this study, results had been shown that the increased BTP concentrations in the serum of patients with CKD highly significantly correlated with the concentrations of creatinine, cystatin C. BTP had a higher value of correlation with mGFR among other endogenous biomarkers (r = 0.799). BTP is superior in performance over creatinine and an alternative to cys.C in detecting mild reduction in GFR in children [49]. Results illustrated that cys.C is equal if not superior over serum creatinine as a predictor of renal function [21] (table 6).

Table (6). Pearson correlation test, correlation (r) and P values among GFR and endogenous biomarkers had been taken.

		mGFR	H-BTP	L-BTP	Scr	Scys.C
mGFR	r value*	1	- 0.799*	- 0.166*	- 0.661*	- 0.591*
	P value*		0.000	0.033	0.000	0.000
BTP	r value*	- 0.799*	1	0.209*	0.558*	0.583*
	P value*	0.000		0.003	0.000	0.000
Scr	r value*	- 0.661*	0.558*	0.112	1	0.595*
	P value*	0.000	0.000	0.118		0.000
Scys.C	r value*	- 0.591*	0.583*	0.209*	0.595*	1
	P value*	0.000	0.000	0.003	0.000	

*P and r values of Pearson Correlation test

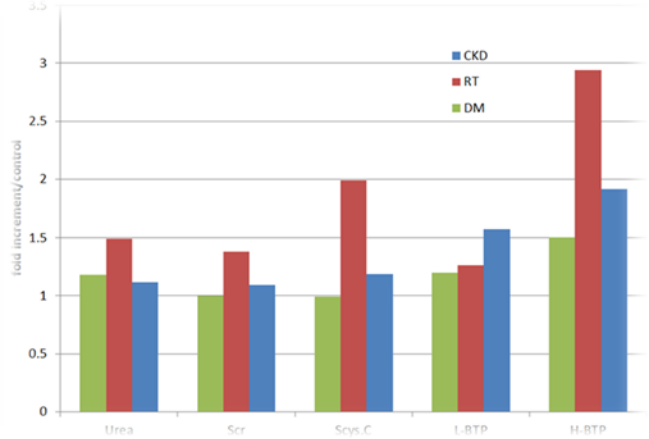

Figure (3). Fold increment of biomarkers in all renal disease groups comparing to control group (mean ratio).

The renal characteristic make measurement of BTP concentration as a useful tool to assess GFR impairment, may be more sensitive than creatinine measurement.[22]

The question that comes to mind is: why we should be confident of the higher level of BTP in general and its stronger correlation with mGFR, at least over the cys.C did?

The answer of this question depends on the advantages of BTP over the cys.C which are:

1. BTP not affected by C-reactive protein or at least, have no significant correlation with C-reactive protein level [23].
2. It is not affected by body composition as cys.C did and less extra renal interferences that considered by some authors more suitable marker [23], and it lack the affinity for protein binding [24].
3. Its concentration not affected by thyroid function which is unlike cys.C that concentration is affected [23].
4. Patients of RT group are normally on corticosteroids therapy, which is affected cys.C concentration and falsely elevates its serum concentration, it had been found that serum level of BTP was not affected by such treatment [25].
5. During hemodialysis, BTP not affected an so not removed. So, it can consider as an advantage to be added to regard BTP as an endogenous filtration marker for GFR impairment [26].
6. Some authors had been found that BTP level is not superior over cys.C for GFR assessment and there was no significant differences between them [27], although results done by manzano Fernandez et al (2011) had been illustrated that BTP is better than cys.C as a prognostic marker. However, results of these studies based on small sample size and that can be consider as a limitation [28].

Serum creatinine Scr has limitations as a marker of kidney function, it is not specific and most notably insensitivity because it remains in the normal reference interval–with a flat slope over much of the GFR range until GFR is decreased about 75% (serum creatinine have an “exponential” relationship with GFR (figure 2). This exponential relationship makes interpretation of changes in serum creatinine difficult) [29-32]. A small (but significant) and variable amount of the creatinine appearing in the urine is derived from “tubular secretion”: about, 7 to 10 % is due to tubular secretion,[17] but this amount is increased in the presence of renal insufficiency. SCr is affected by non-GFR determinant factors as age, gender, muscle mass (source of production) [33], and diet. [13,34] and extra renal clearance in CKD [35].

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