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Original Article

Biochemistry Section

Association of Serum Beta-Trace Protein Levels in Patients with Chronic Kidney Disease: A Case-control Study

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ABSTRACT

Introduction: Chronic Kidney Disease (CKD) is common disorder showing decreased Glomerular Filtration Rate (GFR) value (<60 mL/min/1.73 m^2). Because of limitations of creatinine as a biomarker of GFR, new alternative biomarkers are being investigated, such as Beta-Trace Protein (BTP) is low molecular weight proteins that are filtered by the glomeruli. Serum BTP have been shown to be more helpful for estimating GFR.

Aim: To assess the role of Beta-Trace Protein (BTP) as a potential biomarker of Chronic Kidney Disease (CKD) in comparison to serum urea, serum creatinine, fasting blood sugar and Creatinine Clearance Rate (CCR).

Materials and Methods: This case-control study was conducted at Government Medical College, Rajouri, Jammu and Kashmir, India, from February 2021 to December 2021. Total 50 known patients of kidney diseases and 50 healthy individuals above the age of 18 years were enrolled in the study. Blood samples were collected from all individuals and serum BTP, serum urea level, serum creatinine level, fasting blood sugar were measured. Correlation of BTP with serum urea level, serum creatinine level, Fasting Blood Glucose (FBG) level, and CCR was calculated by Pearson Correlation test.

Results: In present study, 50 patients in case groups (33 male and 17 females) and 50 healthy controls (25 males and 25 females) were included. Among controls, the mean age of patients was 52.12±5.66 years and among cases 55.94±10.51 years. BTP level was increased two times (from 32.06±11.25 µg/mL to 66.36±27.80 µg/mL) in CKD patients than controls individuals. BTP level was positively correlated with serum urea level, serum creatinine level, and FBG level while negatively correlated with CCR.

Conclusion: The 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 creatinine as an alternative endogenous GFR marker.

Keywords: Biomarker, Glomerular filteration rate, Renal dysfunction, Serum creatinine

INTRODUCTION

The CKD is a progressive abnormality of kidney in either structural or functional form affecting greater than 10% of the population worldwide [1]. It is more prevalent among geriatric age group along with other complications such as diabetes, obesity and coronary/vascular disease [1]. Serum creatinine level has been used as basic biomarker for detection of CKD since years but it has many restrictions. The serum creatinine level is varied according to various factors such as age, gender, body weight etc., [2]. Researcher suggested that determination of serum creatinine level is not accurate method to diagnose CKD, therefore there is need of alternate new biomarkers for early detection of CKD with accuracy as well as precision [3].

Various low molecular weight proteins in the range of 10-25 kDa have renal handling compatibility with the "ideal" GFR marker [4]. In fact, they are cleared by the plasma through free glomerular filtration, subsequent complete tubular resorption, and degradation inside tubular cells. As a consequence, their serum concentrations increase progressively with the reduction of GFR. BTP also called lipocalin prostaglandin D_2 synthase is a low molecular weight (23-29 KDa) glycoprotein. It is isolated from cerebrospinal fluid. It is present in very low concentration in blood [5,6].

The BTP may be a promising biomarker. Because of BTP's low molecular weight, it is expressed in almost all tissues involved in prostaglandin metabolism, such as the brain, retina, melanocytes, male reproductive organs, heart and kidney [7-10]. BTP is filtered freely in the kidneys and has very little tubular reabsorption and is excreted in the urine. Few researchers found that BTP serum levels correlate with GFR [11-13]. Therefore, the present investigation was aimed to assess the role of BTP as a potential biomarker of CKD as previously GFR and CCR were considered as biomarkers for CKD. Also, to assess its relation to other biomarkers such as serum urea level, serum creatinine level, CCR, and FBG level.

MATERIALS AND METHODS

This case-control study was conducted at Government Medical College, Rajouri, Jammu and Kashmir, India, from February 2021 to December 2021. Study was approved by Ethics committee (IEC Reference no. AU/EC/FM/2021/187), Adesh University, Bathinda. Written informed consent was obtained from all the subjects prior to the study.

Inclusion criteria: Patients aged >18 years, with established diagnosis of CKD i.e., whose GFR levels were between 59-15 mL/min per 1.73 m^2 were included. Healthy individuals aged >18 years were taken as control.

Exclusion criteria: Subjects with primary tubular diseases, Recent or concurrent administration of potentially nephrotoxic drugs, acute kidney injury, terminal kidney failure requiring dialysis were excluded from the study.

Fifty patients of known CKD were selected as cases from GMC Rajouri by convenient sampling. Fifty healthy individuals working in GMC Rajouri were selected as controls.

Study Procedure

The demographic data such as age, gender, height, weight, Body Mass Index (BMI), blood pressure was collected from all the study subjects. Under aseptic precautions, 5 mL of venous blood sample was collected after an overnight fasting of 12 hours from all subjects. After retraction of the clot, samples were centrifuged at 2000 rpm for 15 minutes for separation of serum. Serum was divided into two parts in aliquotes at -20°C for the estimation of BTP. The remaining serum was used for estimation of urea, and creatinine

level. For estimation of glucose, separate blood sample was drawn in tube containing fluoride as gycolytic inhibitor and plasma sample was used.

Estimation of serum Beta-Trace Protein (BTP) (Normal value 0.78-50 ng/mL): Determination of concentration of BTP in serum was measured by Enzyme Linked Immunosorbent Assay (ELISA). Enzyme immune assay kit was purchased from bio vendor Aviva System Biology Corporation. An antibody specific for PTGDS (Prostaglandin-Endoperoxide Synthase) had been precoated onto a well plate. To wells, test samples were incubated for 30 minutes. In the wells, biotinylated detector antibodies were added which were specific for PTGDS. The mixture was incubated and then washed. After that, Avidin peroxidase conjugate was added. After incubation, unbound conjugates were washed away. TMB substrate (3,3,5,5-tetramethylbenzidine) was added for enzymatic reaction through which blue coloured product was formed which produced yellow colour after addition of acidic stop solution. The intensity of yellow colour was measured at 450 nm [14].

Estimation of urea (Normal value 19-45 mg/dL): For determination of serum urea level, Urease-Glutamate Dehydrogenase (GLDH) method was used [15]. All reagents (Urea standard, Tris buffer pH 8.5, a-ketogluterate GLDH, urease, and Nicotinamide Adenine Dinucleotide Hydrogen(NADH)) were purchased from sigma Aldrich. A 1 mL of working solution and 10 µL of standard/sample was mixed and incubated for 30 seconds and absorbance was measured at 340 mm (A_1). Exactly after one minute, absorbance was measured at 340 nm for both standards and test (A_2). Urea concentration was calculated by using following formula:

Urea concentration=Absorbance of sample (A1-A2)×Standard value

Absorbance of Standard (A1-A2)

Estimation of serum creatinine (Normal range-in male 0.7-1.3 mg/dL; in females- 0.6-1.1 mg/dL): Creatinine level was estimated by the Jaffe's method, used with slight modifications [16]. In 1 mL of sodium tungstate reagent, 1 mL sulfuric acid reagent, and 1 mL of distilled water was added. After mixing, 1 mL of serum sample was added. The mixture was centrifuged at 1500 rpm for 5 minutes. In the filtrate, picric acid reagent and NaOH sequentially to all tubes after all other materials have been added. After mixing, tubes were allowed to stand for 15 minutes at room temperature. Absorbance was measured at 510 nm. Creatinine value for test by using the concentration of the standard and its absorbance.

Determination of CCR: Creatinine clearance can be estimated using serum creatinine levels. The Cockcroft-Gault (C-G) formula uses a patient's weight (kg) and gender to predict CrCl (mg/dL). The resulting CrCl is multiplied by 0.85 if the patient is female to correct for the lower CrCl in females. The C-G formula is dependent on age as its main predictor for CrCl. Below is the formula:

eCCr=(140-Age)×Mass (kg)×(0.85 if female)/72×{Serum Creatinine (mg/dL)} [17].

Estimation of plasma glucose (normal range- 74-100 mg/dL): For determination of glucose level in blood, Glucose oxidase-peroxidase (GOD-POD) method was used with end point analysis [18]. Absorbance was measured at 505 nm. The intensity of the coloured complex was directly proportional to the concentration of glucose in samples.

STATISTICAL ANALYSIS

The statistical analysis was performed by using Statistical Package for Social Sciences (SPSS) version 26.0. Results were expressed as arithmetic mean and standard deviation (Mean±SD). Differences of continuous variables between the different group's data were assessed by independent samples t-test. Pearson correlation was used to assess the correlation between parameters of interest. The level of significance was observed at p<0.001.

RESULTS

In control group, out of 50 healthy persons 25 were male and 25 female, while for cases, 33 male and 17 females were selected. Among controls, the mean age of patients was 52.12±5.66 years and among cases 55.94±10.51 years. Demographic features like height and BMI of controls were not comparable with cases [Table/Fig-1].

The FBG level was found to be significantly higher in cases (154.77±71.75 mg/dL) than healthy individuals (104.58±9.11). SBP and DBP were also found to be higher in case group as compared to control group. The difference between case and controls regarding FBS, SBP and DBP was found statistically significant [Table/Fig-2].

Results showed that creatinine and urea level in cases was found to be elevated than control. The variation was greater in females than males among case group. The levels of serum urea, Creatinine were significantly higher (p-value <0.001) in cases than control while CCR were significantly lowered (p-value <0.001) in patients with kidney impairment than control. The statistically significant difference (p-value <0.001) was found between control and case groups regarding BTP level, which indicated the increased level of BTP concentrations in case group as compared to controls [Table/Fig-3].

	Mean±SD						
	Control			Case			
Variables	Total	Male	Female	Total	Male	Female	p-value
Gender	50	25	25	50	33	17	0.50
Age (years)	52.12±5.66	53.08±5.56	51.16±5.60	55.94±10.51	57.72±10.74	52.47±9.10	**0.041**
Height (m)	1.61±0.08	1.65±0.09	1.58±0.06	1.66±0.08	1.69±0.06	1.61±0.09	**0.010**
Weight (Kg)	68.08±9.13	70.64±10.16	65.52±7.10	69.3±8.6	70.27±7.64	67.41±10.15	0.15
BMI (Kg/m^2)	25.41±1.97	25.09±1.86	25.73±2.02	24.61±2.65	24.14±2.53	25.53±2.64	**0.05**

[Table/Fig-1]: Demographic data of the cases/controls involved in the study.
Chi-square test, independent t-test; BMI: Body mass index; Bold p-values are significant

	Mean±SD						
	Control			Case			
Variables	Total	Male	Female	Total	Male	Female	p-value
FBG (mg/dL)	104.58±9.11	104.45±8.37	104.72±9.79	154.77±71.75	155.96±69.01	152.47±76.74	**<0.001**
SBP (mmHg)	119.16±6.57	120.72±6.66	117.6±6.09	136.36±25.65	133.75±10.47	141.41±22.92	**0.014**
DBP (mmHg)	78.56±3.89	79.76±2.35	77.36±6.09	87.68±9.97	87.21±10.47	88.58±8.83	**0.006**

[Table/Fig-2]: Fasting Blood Glucose (FBG), Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) in cases/controls involved in the study.
Independent t-test

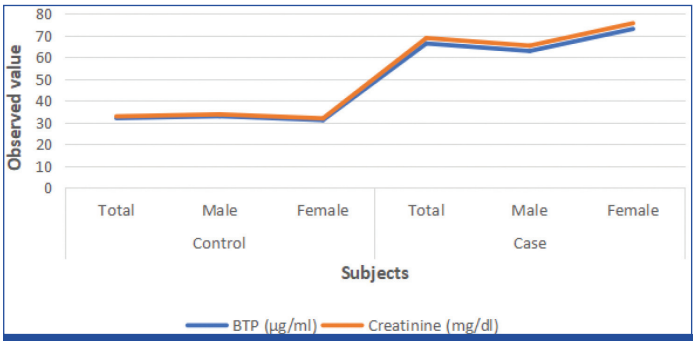
Variables	Mean±standard deviation						p-value
	Control			Case			
	Total	Male	Female	Total	Male	Female	
BTP (µg/mL)	32.06±11.25	32.96±12.46	31.166±9.80	66.36±27.80	62.90±27.54	73.05±27.07	**<0.001**
Creatinine (mg/dL)	0.83±0.12	0.85±0.11	0.81±0.12	2.51±1.16	2.46±1.10	2.62±1.27	**<0.001**
CCR (mL/min)	111.34±10.34	114.73±8.46	107.96±10.93	35.73±13.32	37.67±12.92	31.95±13.26	**<0.001**
Urea (mg/dL)	28.24±6.82	28.47±6.36	28.02±7.24	190.55±44.52	88.91±40.01	93.74±52.03	**<0.001**

[Table/Fig-3]: Observations of Beta-Trace Protein (BTP), creatinine, Creatinine Clearance Rate (CCR), and urea in cases/controls involved in the study.
Independent t-test; bold p-values are significant

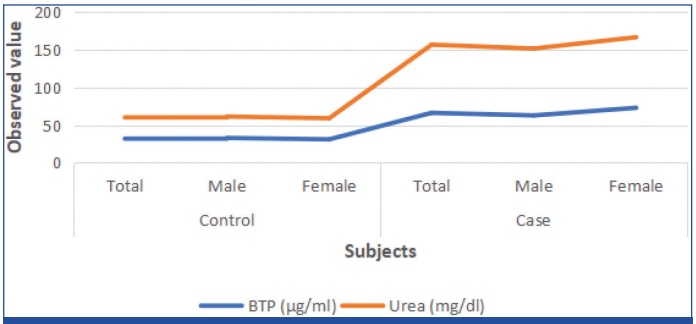
In patients with renal impairment, a strong positive correlation was found for serum creatinine and urea level with BTP (r value=0.99 for both). While BTP was negatively correlated with CCR (r=-0.98 for both). The correlation of BTP with serum urea level, serum creatinine level, CCR and FBG was found statistically significant (p-value <0.001) [Table/Fig 4-8].

Analysis	Creatinine	Urea	CCR	FBG
Pearson correlation	0.99	0.99	-0.98	0.97
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**

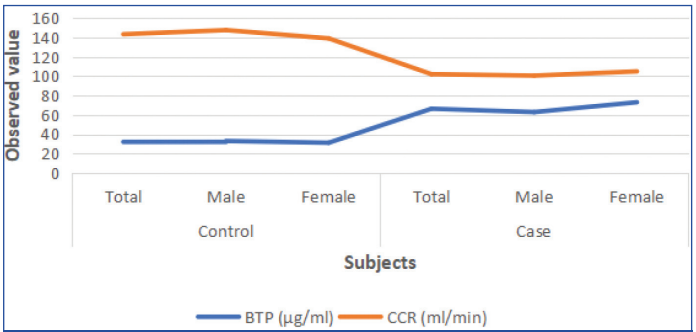
[Table/Fig-4]: Pearson correlation of Beta-Trace Protein with Creatinine, urea, Creatine Clearance Rate (CCR), and Fasting Blood Glucose (FBG).
Correlation is highly significant at 0.01 level; bold p-values are significant



[Table/Fig-5]: Pearson correlation between BTP and creatinine.



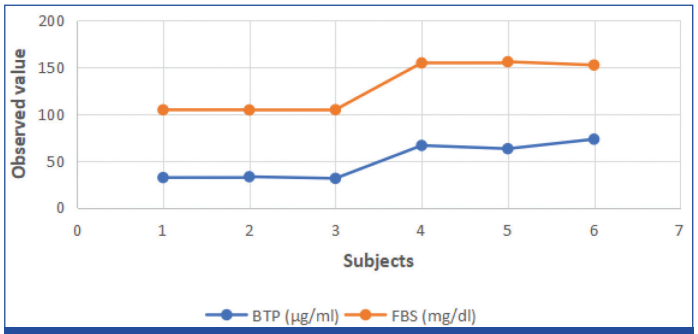
[Table/Fig-6]: Pearson correlation between BTP and Urea.



[Table/Fig-7]: Pearson correlation between BTP and CCR.

DISCUSSION

Beta-trace protein may be a good biomarker for diagnosis of CKD. In the present study, it was found that serum level of beta-trace protein was significantly higher in case group of study than control group. Kidneys play important roles in body to perform various vital functions such as removal of excessive water and waste products from body, and regulation of acid-base balance of the blood. In CKD, kidneys are unable to perform its vital functions properly. CCR is considered as good indicators of any damage of kidneys (either structural or functional [19].



[Table/Fig-8]: Pearson correlation between BTP and FBG/FBS.

Now-a-days, CKD has become chronic health issues worldwide. CKD is considered as the twelfth most common cause of death. Its mortality rate is ~31.7% which is a serious health concern [20,21]. Side-effects associated with CKD can be reduced if it is diagnosed at early stage. So, in the present investigation, concentration of BTP in serum was aimed to find out its efficacy for early detection of renal dysfunction in patients of CKD.

Creatinine and urea are considered as good biomarkers to diagnose CKD [22-24]. Results of the present study showed that in patients of CKD, serum level of urea and creatinine was elevated by approximately three times. However, the elevation in serum urea level and serum creatinine level was 218.05% and189.41%, respectively in males while in female groups this increment was found to be higher (234.54% and 223.45%, respectively). Thus, the elevation was higher in female patients than male. CCR was lowered in patients with renal impairment than control. These findings were in accordance to some previous findings which have concluded that the increased serum creatinine has a renal cause and consider a result of reduced CCR which is also related to increased serum urea concentration [25-27].

The FBG was also measured in all subjects involved in the study and results revealed that in patients with renal impairment values are higher than healthier persons. Results indicated the association of CKD with hyperglycaemia. Results clearly conclude that BTP was increased approximately two-folds (106.98 %) in patients with kidney impairment than healthy individuals. However, variation was higher in female groups (134.43%) than male groups (90.83%). These findings were in agreement with the study of Bishoy EA et al., [28]. They have also reported that beta-trace protein have been elevated in patients with various renal diseases.

Findings of the current investigation also showed that concentration of BTP was positively correlated with serum urea level, serum creatinine level and FBG level while negatively correlated with CCR. These conclusions were in consistent with the previous studies, which stated that elevated beta-trace protein concentrations in CKD patients have significantly positive correlation with concentration of creatinine [29,30]. Hence BTP is good marker for early diagnosis of renal dysfunction in patients with CKD.

Limitation(s)

Due to limitations of present study, namely small sample size and heterogeneity of filters, additional systematic investigations are warranted to further elucidate the value of BTP as a diagnostic tool in patients on CKD.

CONCLUSION(S)

The BTP level showed an elevation in serum of patients with renal impairment. BTP concentration in serum showed strong positive correlation with FBG, serum urea level, and serum creatinine level while it showed strong negative correlation with CCR. Present study suggests Serum BTP might me a potential biomarker for patients of CKD. However, some more studies are recommended in future for validation of the results.

Authors contribution: This study was conducted by MT under supervision of author RSA and the author GKB helped for designing the study.

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