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Association of Biochemical Parameters with Renal Functions of End Stage Renal Disease (ESRD) Patients of Bangladesh

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Abstract

Biochemical parameters are greatly influenced by genetic, physiological and environmental factors. Regional data is essential to establish a relationship between End stage renal disease (ESRD) and biochemical parameters. In Bangladesh, there is not any established research data on biochemical parameters of ESRD patient. The present study was a case control study with 500 ESRD patients and equal number of healthy volunteers (controls). Sociodemographic, anthropometric and clinical data of both patients and controls were collected. Serum biochemical parameters were analyzed by laboratory test. Statistical software package SPSS were used for independent sample t-test and Pearson's correlation test. Our study found that serum creatinine, blood urea nitrogen (BUN) and serum uric acid were significantly higher (p<0.05) and serum potassium were significantly lower in ESRD patient as compared to control subjects (p<0.05). But serum chloride, serum sodium and serum calcium level were in the normal range. Pearson correlation natlysis reveals that serum creatinine and serum chloride was inversely correlated with GFR (glomerular filtration rate) in both patient and control groups. This study explored that ESRD patients have higher level of serum potassium than normal patient. Routine investigation of serum biochemical parameters may help to prevent ESRD complication.

Keywords: Serum creatinine; Blood urea nitrogen (BUN); End Stage Renal Disease (ESRD)

Introduction

ESRD or end stage renal disease means established kidney failure when GFR<15 ml/min/1.73 m² and need permanent renal replacement therapy or dialysis [1]. Chronic kidney disease (CKD) affects about 10% of the population worldwide and millions of them are dying every year due to lack of treatment [2]. CKD was ranked 27th in the list of causes of total number of deaths worldwide in 1990, but rose to 18th in 2010 according the 2010 Global Burden of Disease Study. Among all patients more than 80% who receive treatment for kidney failure are in affluent countries with universal access to health care and large elderly populations. Number of cases of kidney failure will increase disproportionately in developing countries, such as China and India, due to increasing number of elderly populations [3]. Currently over 2 million people receive treatment with dialysis or a kidney transplant to stay alive worldwide, yet this number may only represent 10% of people who are actually need treatment to live. Of the 2 million people receiving treatment for ESRD, the majority are treated in only five countries - the USA, Japan, Germany, Brazil and Italy. But these five countries represent only 12% of the world population. In about 100 developing countries that make up over 50% of the world population, there only 20% of ESRD patient are treated [4].

The number of CKD patients with End Stage Renal Disease (ESRD) is increasing drastically day by day. The treatment procedure of ESRD includes dialysis or kidney transplantation [5]. In developed countries, the treatment of ESRD comprises 2%-3% of total healthcare expenses, while only ESRD patients represent 0.02-0.03% of the total population [6]. In 2012, the expenditure for ESRD is \$32.9 billion which is reported by The United States Renal Data System where inpatient services cost 38%, outpatient care cost 34%, physician/supplier costs 21% and drugs

J Bioanal Biomed, an open access journal ISSN: 1948-593X cost 2% [7]. Around 40, 000 people reportedly die each year from CKD in Bangladesh. Around 20, 000-30, 000 patients suffer from acute kidney failure [8]. Among the kidney patients 100-120 patients approximately reach ESRD in Bangladesh every year [9].

Though infections of kidney disease are considered less important in the western world [10] but currently obesity, diabetes [11,12] and hypertension [13,14] are indicated as the prime considerations of kidney disease. Cardiovascular disease is also the risk factor for ESRD and sometimes vice versa [15-17]. Again, age is a key predictor of ESRD and 11% patients without hypertension and diabetes are at stage 3 or worse CKD who are older than 85 years [18]. According to the sex, males with pre-existing diabetes, hypertension and CKD of Asia, Europe, Australia, Africa-Americans and developing countries such as Africa, China and India are at higher risk for End-Stage Renal Disease (ESRD) [19]. One study revealed 16% prevalence rate of CKD occurs in slums of Dhaka. The percentage was found which belongs to stage-1, stage-2, stage-3,

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stage-4 and stage-5 diseases are respectively 1.3%, 3.4%, 11%, 0.3% and 0.1% [20]. Another study revealed 17.4% prevalence rate of CKD in Savar, Dhaka. And the percentage was recorded to belong to stage-1, stage-2, stage-3, stage-4 and stage-5 (ESRD) diseases are respectively 1.7%, 2.1%, 13.1%, 0.3% and 0.2% [21]. As dialysis and kidney transplantation are very expensive so early detection and intervention are the best strategies for ESRD treatment [16].

The present study is undertaken to evaluate the estimation of renal function of patients with end stage renal disease (ESRD) and also to investigate the correlation between the studied biochemical parameters.

Materials and Methods

Study design and data collection

The study protocol was approved by the ethical approval committee of Kidney Foundation Hospital and Research Institute, Dhaka, Bangladesh. This was a case-control study carried out between January, 2016 to December, 2016 consisting of 500 patients with ESRD and 500 healthy volunteers as controls. Demographic data was collected with a well-designed questionnaire by regularly attending in Kidney Foundation Hospital and Research Institute and clinical data were obtained by lab analysis. Before data collection, we conducted a pilot study with a few patients by structured questionnaires to set the variables of the study. According to the purpose of the study, the necessary modification was done in the questionnaires before conducting the final study. Ethical clearance was obtained from the ethical review board of the institution. The purpose of the survey was informed to each subject prior inclusion into the study. Respondents were included in the study having a high level of creatinine than normal and GFR \leq 15 ml/min/1.73 m². The healthy volunteer creatinine level will be more than 90 ml/min/1.73 m². The patients who disagreed to donate blood were excluded from the study.

Blood sample collection

5 mL venous blood sample using a plastic syringe fitted with a sterile stainless steel needle was collected from each patient and control. Before collecting blood, each patient did overnight fasting which is about eight hours. Collected blood samples were taken in a metal-free sterile tube, allowed to clot at room temperature for half an hour and then centrifuged at 3000 rpm for 15 min to extract the serum. The extracted serum was taken in an eppendorf, analyzed for serum ions and creatinine immediately. To avoid the possible interference in the test reading, all the steps were performed in dust-free environment.

Chemicals and reagents

The chemicals used for the study were of analytical grade from commercially available company.

Analytical procedure

A series of coupled enzymatic reactions were carried out to assess the creatinine level including creatininase enzymatic conversion of creatinine into the product creatine which itself is converted to sarcosine by creatineamidinohydrolase (creatinase), followed by oxidation of sarcosine by sarcosine oxidase (SOD) producing hydrogen peroxide. Finally the hydrogen peroxide was quantified at 550 nm by the formation of a colored dye in the presence of peroxidase. All measurements were performed using an Olympus AU 400 analyzer [22]. Serum sodium, potassium and chloride were measured by ion selective electrode (ISE) method in the Beckman Coulter AU auto analyzer [23-25]. Blood urea nitrogen was assessed by enzymatic methods where the enzyme urease converts urea to ammonia and carbonic acid, which are proportional to the concentration of urea in the sample [26]. Estimation of Serum uric acid was done by uricase method [27]. Serum calcium was estimated by the nephelometric method of Lyman [28].

Statistical analysis

Statistical analysis was performed using statistical software package SPSS version 22.0 (SPSS, Inc., Chicago, IL). All data were expressed as mean \pm standard error mean (mean \pm SME). Pearson's correlation analysis was used to find the interrelation between the various biochemical parameters.

Results

Anthropometric and clinical characteristics of subjects

The mean value of systolic and diastolic blood pressure (SBP and DBP respectively) were $150.23 \pm 1.89 \text{ mmHg}$ and $84.43 \pm 1.19 \text{ mmHg}$ for patient group and $130.00 \pm 1.84 \text{ mmHg}$ and $83.05 \pm .976 \text{ mmHg}$ for the healthy control group respectively. The average GFR (Glomerular filtration rate) was calculated 6.90 ± 1.62 and 98.07 ± 3.16 in patient and control groups respectively which has been indicated in Tables 1 and 2. Sociodemographic data is given below.

Different serum composition profile

The serum creatinine level was found 872.17 \pm 20.17 µmol/L and 77.89 \pm 1.69 µmol/L in patient and control groups respectively. The BUN was 31.42 \pm 1.38 mmol/L and 6.08 \pm 1.09 mmol/L in patients and control groups respectively. The serum uric acid, serum sodium, serum chloride, serum potassium, serum calcium was found 506.51 \pm 7.89, 132.74 \pm .32, 98.98 \pm .39, 2.05 \pm .05, 2.25 \pm .05 µmol/L respectively in

Variables	Patient group	Control group	
Age years (Mean ± SEM)	51.60 ± 1.39	49.38 ± 1.24	
Sex			
Male	270	230	
Female	230	215	
Area of residence			
Rural	155	125	
Urban	345	375	
Taking high amount of meat			
Yes	280	70	
No	220	430	
Taking high amount of pain killer			
Yes	210	35	
No	290	465	
Taking of antihypertensive drug		5	
Yes	160	50	
No	340	450	
Occupation			
Unemployed	40	25	
Farmer	40	45	
Service	50	20	
Business	120	160	
Housewife	250	250	
Education			
Illiterate	200	190	
Primary	160	180	
Secondary	100	110	
Higher study	40	20	

Table 1: Sociodemographic data of ESRD and control patient.

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patient and 303.05 ± 7.01 , $140.15 \pm .30$, $103.21 \pm .31$, $3.97 \pm .04$ and $2.27 \pm .016 \mu$ mol/L in control groups respectively (Table 3).

To establish correlation between different parameters these data were further analyzed. The correlation was established between GFR, BUN and creatinine with different parameters (Table 4). The statistical data showed a negative correlation between SBP and GFR (r=-0.091*, p=0.039), DBP and GFR (r=-0.118**, p=0.008), serum creatinine and GFR (r=-0.753**, p=0.000), BUN and GFR (r=-0.217**, p=0.000), serum uric acid and GFR (r=-0.142, p=0.000), serum sodium and BUN (r=-0.011, p=0.797), serum chloride and GFR (r=-0.057, p=0.197), serum potassium and GFR (r=-0.300, p=0.002), serum potassium and GFR (r=-0.264**, p=0.000), serum calcium and GFR(r=-0.023, p=0.605), serum sodium and creatinine (r=-0.030, p=0.494), Serum calcium and creatinine (r=-0.048, p=284), Age and creatinine (r=-0.235, p=0.000), Age and BUN (r=-0.016, p=0.720) but a positive correlation between SBP and BUN (r=0.025, p=0.578), DBP and BUN (r=0.053, p=0.234), Serum creatinine and BUN (r=0.234**, p=0.000), serum uric acid and BUN (r=0.107^{*}, p=0.016), serum sodium and GFR (r=0.005, p=0.906), serum chloride and BUN (r=0.012, p=0.794), serum potassium and BUN (r=0.029, p=0.517), serum calcium and BUN (r=0.270**, p=0.000), SBP and creatinine (r=0.026, p=0.559), DBP and creatinine (r=0.152**, p=0.001), BUN and creatinine (r=0.234**, p=0.000), serum uric acid and creatinine (r=0.179**, p=0.000), serum chloride and creatinine (r=0.061, p=0.166), Serum potassium and creatinine (r=0.230**, p=.000) Age and GFR (r= 0.114^{**} , p=0.010) in patient group.

Discussion

Several international studies conducted in Japan, France, Iran and Korea shows that ESRD dramatically increases with aging particularly after the age of 50 in both genders and males develop ESRD more than females [29-31].

In our study we found that there is a negative correlation between age and GFR which means that ESRD is increased with age because with aging GFR is decreased.

One study showed that there is a significant association between patients who have diabetes mellitus and hypertensive with the onset of ESRD. This can be narrated on the light of rapid urbanization, transformation into sedentary life style, increase in prevalence and incidence in diabetes mellitus and hypertension. When a patient suffers from theses chronic disease (diabetes mellitus, hypertension

Parameters	Reference value (mmHg)	Patient group	Control group	P-value
SBP (mm Hg)	<140	150.23 ± 1.89	130.00 ± 1.84	<0.05
DBP (mm Hg)	<90	84.43 ± 1.19	83.05 ± .97	<0.05
GFR (ml/min/1.73m ²)	>90	6.90 ± 1.62	98.07 ± 3.16	<0.05
p<0.05 when compare	d to control			

Table 2: Clinical data of study population.

Parameters	Reference value	ESRD patient	Control group	P-value
Serum creatinine (µmol/L)	63.6-110.5	872.17 ± 20.17	77.89 ± 1.69	<0.05
BUN (mmol/L)	3.2-7.4	31.42 ± 1.38	6.08 ± 1.09	<0.05
Serum uric acid (µmol/L)	210-420	506.51 ± 7.89	303.05 ± 7.01	<0.05
Serum sodium (µmol/L)	135-146	132.74 ± .32	140.15 ± .30	<0.05
Serum chloride (µmol/L)	97-106	98.98 ± .39	103.21 ± .31	<0.05
Serum potassium (µmol/L)	3.5-5.3	2.05 ± .05	3.97 ± .04	<0.05
Serum calcium (µmol/L)	2.1-2.55	2.25 ± .05	2.27 ± .01	<0.05

Table 3: Serum composition profile of the study population.

Parameter	Patient group		Control group	
	r	р	r	р
SBP and GFR	-0.091 [*]	0.039	-0.097	0.335
SBP and BUN	0.025	0.578	-0.025	0.808
DBP and GFR	-0.118**	0.008	0.073	0.469
DBP and BUN	0.053	0.234	0.036	0.725
Serum creatinine and GFR	-0.753**	0.000	-0.240 [*]	0.016
Serum creatinine and BUN	0.234**	0.000	0.398**	0.000
BUN and GFR	-0.217**	0.000	0.538**	0.000
Serum uric acid and GFR	-0.142**	0.000	0.057	0.557
Serum uric acid and BUN	0.107*	0.016	0.100	0.322
Serum sodium and GFR	0.005	0.906	-0.089	0.378
Serum sodium and BUN	-0.011	0.797	-0.033	0.744
Serum chloride and GFR	-0.057	0.197	-0.061	0.549
Serum chloride and BUN	0.012	0.794	0.058	0.564
Serum potassium and GFR	-0.264**	0.000	0.037	0.713
Serum potassium and BUN	0.029	0.517	0.137	0.175
Serum calcium and GFR	-0.023	0.605	0.131	0.194
Serum calcium and BUN	0.270**	0.000	0.132	0.192
SBP and creatinine	0.026	0.559	0.013	0.896
DBP and creatinine	.152**	0.001	-0.098	0.331
BUN and creatinine	.234**	0.000	0.398**	0.000
Serum uric acid and creatinine	.179**	0.000	0.242*	0.015
Serum sodium and creatinine	030	0.494	0.058	0.568
Serum chloride and creatinine	.061	0.166	0.168	0.096
Serum potassium and creatinine	.230**	0.000	0.167	0.096
Serum calcium and creatinine	048	0.284	-0.086	0.396
Age and GFR	0.114**	0.010	-0.100	0.322
Age and creatinine	-0.235**	0.000	0.252*	0.011
Age and BUN	-0.016	0.720	0.087	0.389

"indicates p<0.01, indicates p<0.05 when compared to control

Table 4: Correlation between BUN, GFR and creatinine of patient and control group.

and cardiovascular diseases) are more likely to get kidney failure. Changes in the style of living, increase in incident chronic diseases lead to a high complication. There is a significant association between Glomerulonephritis and other urinary tract infection with incident of ESRD which was confirmed by several studies conducted in different countries [32-36]. The associations of different cut-off values of serum creatinine with the risks of death from any causes, particularly with the cardiovascular events have been examined in several studies [37-41]. Most but not all of these studies [42,43] have found increased risks of cardiovascular disease with higher serum creatinine levels. Since serum creatinine levels are not linearly associated with GFR [44], the predictive equations such as the Cockcroft–Gault equation for creatinine clearance [45] and the MDRD equation for estimated GFR [46] has been proposed as a more accurate means of estimating the GFR in populations [18].

Relatively very few studies have evaluated the risk of outcomes in the general population. One research report showed that there is 68% percent increase in the risk of death from any cause associated with an estimated GFR of less than 70 ml/min/1.73 sq. m, as compared with an estimated GFR of at least 90 ml/min/1.73 sq. m. [47]. In our study, we have found that GFR is associated with serum creatinine level. There is a negative correlation between serum creatinine and GFR both in patient and control groups. The patient group had higher creatinine level and lower GFR but the control group had lower serum creatinine level and higher GFR (more than 90 ml/min/1.73 sq. m).

Diabetes and hypertension are the leading causes of ESRD in all developed and many developing countries. But in countries of Asia and sub-Saharan Africa, glomerulonephritis and unknown causes of CKD are more common. The burden of disease moving away from infections towards chronic lifestyle-related diseases increased life expectancy and decreased birth rates in developed countries [48]. In contrast, inadequate supply of safe water, secondary to poor sanitation and high concentrations of disease-transmitting vectors are the key factors for the outgrowth of ESRD in low-income countries [49]. The burden of ESRD in developing countries is also contributed by environmental pollution, pesticides, analgesic abuse, herbal medications and use of unregulated food additives [50]. Due to the acceleration of globalization and urbanization the transition of ESRD have accelerated in the South Asian and Latin American countries together with and increasing prevalence of lifestyle disorders, such as diabetes and hypertension [51,52]. Studies showed that 42% of CKD cases occurred in patients without diabetes while 39% of cases occurred in those patients with undiagnosed diabetes or pre-diabetes. A substantial proportion of adults with undiagnosed diabetes had evidence of kidney disease and kidney function decline [53].

In our research we found that there is a relationship between hypertension and kidney disease. There is a positive correlation between SBP and creatinine and negative correlation between SBP and GFR on kidney patient. So it indicates that there is a close relationship with SBP and kidney disease. Positive correlation between SBP and serum creatinine means the higher serum creatinine level leads to higher SBP while negative correlation between SBP and GFR means the higher SBP is the result of lower GFR. So hypertension is a vital cause for kidney disease.

The standard limit of serum potassium is believed to arise only at the end stage of CKD [54]. Furthermore the linear relationship between potassium and parathyroid hormone (PTH) observed that excess PTH increases basal levels of cytosolic calcium. Cytosolic calcium affects the permeability of the cellular membrane to potassium. So that extra renal disposal of potassium is decreased in case of kidney disease [55]. Potassium stimulates the pancreas to release insulin [56], which is an important regulator of extra renal disposition of potassium. Since glucose-induced insulin secretion is impaired by secondary hyperparathyroidism (SHPT) of CKD [57], which is likely to interfere with potassium-induced insulin secretion. So it could result in the derangement of the extra renal disposition of potassium. Due to impaired intestinal absorption and phosphate retention, serum calcium levels were found to be significantly lower in the cohorts with ESRD than in controls, a finding that is consistent with previously reported observations [58]. Furthermore, as compared to the controls, the kidney patients were about 8 times at risk of developing hypocalcaemia. Excess PTH secretion was stimulated by hypocalcaemia [59].

In our study we found that there is a negative correlation between serum potassium and GFR, positive correlation between serum potassium and creatinine in the patient group. But there is a positive correlation between serum potassium and GFR in control group. Here we understand that after ESRD the serum potassium is decreased and for that reason GFR is increased. We also found a negative correlation between serum calcium and creatinine both in patient and control group. Hypocalcaemia can cause higher serum creatinine level. Therefore hypocalcaemia is also dangerous for the ESRD patient.

Conclusion

Our study explored that ESRD patient have high level of serum creatinine, blood urea nitrogen, serum uric acid and the low level of serum potassium than the healthy control subjects, all of which may contribute worsening of kidney problem. We thus recommend routine investigation of biochemical parameters of ESRD patient specially serum creatinine to prevent complications of chronic kidney disease.

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Competing Interests

The author reports that they have no conflict of interests in this work.

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