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Association between serum bilirubin and estimated glomerular filtration rate in diabetic patients with chronic kidney disease

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Abstract

Background and objectives: Hyperglycemia induces oxidative stress in diabetic patients by increasing reactive oxygen species production, which ultimately damage the cells and cause micro and macrovascular complications including diabetic nephropathy. Increased serum bilirubin level, within physiological range, can inhibit oxidative stress; thereby, preventing development of diabetic nephropathy. The aim of this study was to find out association between serum bilirubin and estimated glomerular filtration rate (eGFR) in diabetic patients with or without chronic kidney disease (CKD).

Materials and method: Both male and female participants aged 30 to 60 years were enrolled in the study. Enrolled participants included healthy individuals (Group-1), diabetic patients without CKD (Group-2) and diabetic patients with CKD (Group-3). Clinical and biochemical parameters namely blood pressure, body mass index (BMI), fasting blood glucose (FBG), HbA1c, eGFR, serum bilirubin and spot urine ACR were measured by appropriate methods. Pearson's correlation coefficient, ANOVA and multiple linear regression models were used to analyze the data.

Result: Total 189 respondents were enrolled in 3 study groups. Each group consisted of 63 cases. Of the 63 cases in Group-3, 49 and 14 belonged to CKD stage 3 and stage 4 respectively. The mean (± SD) serum bilirubin levels of healthy individuals, diabetic patients without CKD and diabetic patients with CKD were 0.66 ± 0.31, 0.64 ± 0.21, 0.46±0.18 mg/dL respectively. Mean serum bilirubin was significantly low ($p<0.001$) in diabetic patients with CKD compared to healthy and diabetics without CKD. A Stepwise multiple regression analysis using eGFR as an objective variable adjusted for risk factors as explanatory variables, showed that serum bilirubin ($\beta=0.323$, $p<0.001$) was significantly associated with eGFR, in addition to age, BMI, HbA1c and urinary ACR.

Conclusion: The study has demonstrated that low serum bilirubin level is associated with CKD in diabetic patients and it could be used as a simple marker for CKD in diabetics.

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Introduction

Chronic hyperglycemia in diabetes is associated with long term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1,2]. Chronic kidney disease (CKD) is a frequent long-term complication of diabetes. CKD is a leading cause of end-stage kidney disease in diabetics, accounting for 50% of cases [3]. It is characterized by persistently elevated urinary albumin excretion (albumin-to-creatine ratio [ACR] ≥ 30 mg/g) and/or low estimated glomerular filtration rate (eGFR < 60 mL/min/1.73 m^2) in a person with diabetes [4].

Oxidative stress has been considered a pathogenic factor for the development of nephropathy in diabetic patients [5,6]. Bilirubin is a potent antioxidant and it largely protects cells against lipid peroxidation [7]. It is generated from biliverdin by biliverdin reductase. During its antioxidant activity, it is oxidized to biliverdin which is immediately reduced again by biliverdin reductase to bilirubin [8].

The precise nature of the relationship between serum bilirubin level and development of nephropathy in diabetic patients is unknown. But it is expected that increased serum bilirubin level within physiological range can inhibit oxidative stress and inflammation; thereby, preventing development of diabetic nephropathy [9]. Previous studies reported that low serum bilirubin level predicts the development of chronic kidney disease in patients with type 2 diabetes mellitus [10]. But with the best of our knowledge there is no study regarding the relationship of serum bilirubin and eGFR in diabetic patients with or without CKD in Bangladesh. So, this study aimed to find out association between serum bilirubin and eGFR in diabetic patients with or without CKD in our population.

Materials and method

The study was conducted at the Department of Biochemistry and Molecular Biology, BIRDEM General Hospital over one year period.The study was approved by Institutional Review Board, BIRDEM. Informed written consent was obtained from each participant prior to the enrollment in the study.

Both male and female diabetic patients with and without CKD and between the age group of 30 to 60 years were selected from outpatient department of Medicine, BIRDEM General Hospital and enrolled in the study. Patients with jaundice, acute kidney injury, kidney disease with non-diabetic etiology or patients on renal replacement therapy were excluded. Also pregnant women, patients taking nephrotoxic or hepatotoxic drugs were also excluded.

Detail clinical and biophysical characteristics of each participant were recorded in a structured questionnaire. Diabetes mellitus was diagnosed based on WHO criteria [11]. CKD was diagnosed on the basis of persistent albuminuria (>30mg/day or ACR>30mg/g) in at least two occasions within six months period and/or GFR less than 60 ml/min/1.73m^2 for more than three months [12]. Estimated GFR was calculated by CKD-EPI method. Serum creatinine was measured in Jaffe's method by Abbott ARCHITECT PLUS C 8000 Autoanalyzer. Serum bilirubin was measured by photometric method in Abbott ARCHITECT PLUS C 8000 Autoanalyzer. HbA1c was measured by High Performance Liquid Chromatography (HPLC) method by BIO-RAD Variant TM II Turbo. Spot urine microalbumin (mg/L) was measured in particle-enhanced turbidimetric inhibition immunoassay and urine creatinine (g/L) was measured by Jaffe's method by SIMENS Dimension EXL 200. Urine microalbumin creatinine ratio (mg/g) was calculated. Hemoglobin was measured by Sodium lauryl sulphate method in SYSMEX XN-1000 Autoanalyzer.

Pearson's correlation coefficient, multiple linear regression analysis and ANOVA tests were done to determine the relation between serum bilirubin and eGFR. All statistical tests were considered at 5% level of significance. SPSS version 22 was used for data analysis.

Results

A total of 189 respondents were included. Out of 189 cases, 63 were healthy individuals (Group-1), 63 were diabetic patients without CKD (Group-2) and 63 were diabetic patients with CKD (Group-3). Cases of Group-3 were further divided according to

the stage of kidney disease. Of the 63 Group-3 cases, 49 and 14 belonged to CKD stage 3 and stage 4 respectively. In Group-1, 2 and 3, 50.7%, 74.6% and 67.7% participants were male respectively.

Table-1 shows the detail clinical and biochemical parameters of the three study groups. Age, systolic and diastolic blood pressure were significantly ($p<0.05$) higher in diabetic patients with CKD than other two groups. Mean BMI was significantly ($p<0.05$) higher in patients of Group-3 in comparison to Group-1 and 2. Mean hemoglobin was significantly lower ($p<0.001$) in Group-3 than Group-1 and 2. Fasting blood glucose and HbA1c of Group-3 patients were significantly ($p<0.001$) lower than those of other two groups (Group-1 and 2). Estimated GFR was significantly lower ($p<0.001$) in Group-3 cases than those of Group-1 and 2 cases (40.63±13.07, 96.30±18.60 and 78.14±14.51 ml/min/m^2 respectively). Mean serum bilirubin was significantly lower ($p<0.001$) in diabetic patients with CKD (Group-3; 0.46±0.18 mg/dl) compared to healthy (Group-1, 0.64±0.21 mg/dL) and diabetic cases without CKD (Group-2, 0.46±0.19 mg/dL).

Table-2 shows the differences of clinical and biochemical parameters of Group-3 diabetic patients with stage 3 and stage 4 CKD. Mean eGFR was significantly lower ($p<0.001$) in stage 4 CKD patients than stage 3 CKD patients. Serum bilirubin was also found significantly lower ($p=0.029$) in stage 4 CKD patients (0.37±0.10 mg/dl) compared to those of stage 3 CKD patients (0.48±0.19 mg/dl).

Table-1: *Comparison of clinical and biochemical parameters of the three study groups*

Variables	Group-1 (n=63)	Group-2 (n=63)	Group-3 (n=63)	p value
	Mean ±SD	Mean ±SD	Mean ±SD	
Age (years)	45 ± 8.66	48 ±9.18	52 ± 6.93	<0.001
BMI (kg/m^2)	26 ± 2.51	25.7 ± 3.57	27.4 ± 4.11	0.016
SBP (mm of Hg)	114.76 ± 8.20	123.57 ±14.60	129.92 ± 16.96	0.001
DBP (mm of Hg)	74.13±6.13	79.05±7.40	81.03 ± 7.47	<0.001
Hemoglobin (g/dL)	13.14±0.92	13.95±1.26	12.84±1.58	<0.001
FBG (mmol/L)	4.86±0.58	9.07±2.94	10.17±4.33	<0.001
HbA1c (%)	4.70±0.47	7.81±1.83	9.00±2.51	<0.001
eGFR (ml/min/1.73m^2)	96.30±18.60	78.14±14.51	40.63±13.07	<0.001
Urinary ACR (mg/g)	6.70±3.95	11.81±8.11	128.42±122.76	<0.001
Serum bilirubin (mg/dL)	0.66±0.31	0.64±0.21	0.46±0.18	<0.001

Note: BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, eGFR: estimated glomerular filtration rate, ACR: albumin-creatinine ratio.

Table-2: *Comparison of clinical and biochemical parameters of diabetic patients with stage 3 and stage 4 CKD*

Variables	CKD stage 3 (n=49)	CKD stage 4 (n=14)	p value
	Mean ±SD	Mean ±SD	
Age (years)	52.12 ±7.34	52.64 ± 6.41	0.807
BMI (kg/m^2)	27.47 ± 4.27	27.11 ± 3.63	0.779
Hemoglobin (g/dL)	13.02±1.54	12.20±1.58	0.085
FBG (mmol/L)	10.37±4.62	9.46±3.13	0.496
HbA1c (%)	9.30±2.61	7.97±1.97	0.081
eGFR (ml/min/1.73m^2)	45.78±9.80	22.60±3.29	<0.001
Urinary ACR (mg/g)	130.12±128.84	122.42±102.58	0.838
Serum bilirubin (mg/dL)	0.48±0.19	0.37±0.10	0.029

Note: BMI: Body mass index; FBG: fasting blood glucose; eGFR: estimated glomerular filtration rate; ACR: albumin creatinine ratio; p value calculated by student's t test

Table-3: *Relationship between various risk factors including serum bilirubin and estimated glomerular filtration rate in all study subjects (N=189)*

Variables	Pearson's correlation r (p value)	Multiple linear regression analysis	
		Forced method β (p value)	Stepwise method β (p value)
Age (years)	-0.347(<0.001)	-0.158 (0.007)	-0.185 (0.001)
BMI (kg/m^2)	-0.143 (0.049)	-0.098 (0.076)	-0.107 (0.047))
SBP (mm of Hg)	-0.289(<0.001)	-0.077(0.302)	–
DBP (mm of Hg)	-0.260(<0.001)	0.032 (0.663)	–
Hemoglobin (g/dL)	0.203(0.005)	0.096 (0.081)	–
FBG (mmol/L)	-0.431(<0.001)	-0.160(0.150)	–
HbA1c (%)	-0.472(<0.001)	-0.99 (0.400)	-0.238 (<0.001)
Spot urine ACR (mg/g)	-0.515(<0.001)	-0.319(<0.001)	-0.322 (<0.001)
Serum bilirubin (mg/dL)	0.447(<0.001)	0.302(<0.001)	0.323 (<0.001)
R^2	–	0.49 (<0.001)	0.48 (<0.001)

Note: r: Pearson's correlation coefficient; β: standardized coefficient; R^2: multiple coefficient of determination. eGFR was taken as dependent variable whereas others taken as independent variable.

Table-3 shows the relationship between participants' characteristics and eGFR. Serum bilirubin (r= 0.447, p<0.001) along with BMI, systolic and diastolic blood pressure, hemoglobin, fasting plasma glucose, HbA1c and urinary ACR were significantly related with eGFR. Stepwise multiple regression analysis using eGFR as an objective variable adjusted for risk factors as explanatory variables, showed that serum bilirubin (β=0.323, p<0.001) was significantly associated with eGFR, in addition to age, BMI, HbA_1c and urinary ACR.

Discussion

The present study analyzed the relationship between serum bilirubin concentration and eGFR in healthy individuals and diabetic patients with or without CKD.The different clinical and biochemical profiles of the cases of our study groups were similar to the findings of other reported studies [13-15]. Serum bilirubin level was significantly lower in diabetic patients with CKD (p<0.001) than healthy individuals and diabetic patients without CKD. Similar findings were also found in other studies [13,14].

In our study, among the 63 diabetic patients with CKD, 49 were stage 3 CKD and 14 were stage 4 CKD patients. Serum bilirubin was also found significantly lower (p=0.029) in stage 4 CKD patients compared to stage 3 CKD patients (0.37±0.10 *vs*. 0.48±0.19 mg/dL) indicating that level of serum bilirubin was associated with decline of eGFR. Other studies also reported that bilirubin differed in different stages of CKD [16].

In this study, a stepwise multiple linear regression model using eGFR as an objective variable adjusted for risk factors for explanatory variables demonstrated that, serum bilirubin (β=0.323, p<0.001) was positively and independently associated with eGFR along with age (β=-0.185, p=0.001), BMI (β=-0.185,p=0.001), HbA1c (β=-0.238, p<0.001) and spot urine ACR (β=-0.322, p<0.001) in all study subjects. Katohet al., [10] detected positive association between serum bilirubin (β=0.11, p<0.001) with eGFR along with age (β=-0.29, p<0.001) in a cross-sectional study.

The present study had some limitations. The study was conducted for a limited period of time with relatively small population and convenient sampling was used from a single center. Multicenter, longitudinal, population based study with a large sample size and longer duration is recommended for more accurate and reliable results.

In this study, significantly lower serum bilirubin level was observed in diabetic patients with CKD in

comparison with healthy individuals and diabetic patients without CKD. The results suggest that low serum bilirubin level may predict development and progression of CKD in diabetic patients. Therefore, it is concluded that proper glycemic control and screening of serum bilirubin in diabetic patients would be beneficial for early diagnosis and prevention of progression of CKD in diabetic patients.

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Author's contributions

TT designed the protocol, collected patents' data and samples, analyzed the data and wrote the manuscript; GSS supervised and coordinated the study and edited the manuscript. MHSE collected samples, performed biochemical tests and did the statistical analysis; LS collected sample and did biochemical tests; FY and RAR collected the data including history taking and physical examination.

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Conflicts of Interest

The author declares no conflict of interest.

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