

## RELATION OF SERUM BILIRUBIN IN PHYSIOLOGICAL RANGE WITH SPOT URINE ALBUMIN TO CREATININE RATIO AND eGFR AMONG DIABETIC PATIENTS WITH OR WITHOUT NEPHROPATHY

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### ABSTRACT

Recent studies have suggested a potential role of bilirubin as an antioxidant to protect diabetic patients from hyperglycemia-induced oxidative stress thereby preventing diabetic complications. Diabetic nephropathy is a serious microvascular complication which is determined by increased urinary albumin to creatinine ratio and reduced eGFR. The present work aimed to study the relation of the simple biochemical parameter of serum bilirubin level with the estimated glomerular filtration rate (eGFR) and spot urine albumin to creatinine ratio in diabetic patients with or without nephropathy. This cross sectional study was done in the department of Biochemistry and Molecular Biology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) Academy from July 2017 to June 2018. We have taken 63 diabetic patients without nephropathy and 63 diabetic patients with nephropathy. Data about clinical and biochemical parameters (blood pressure, BMI, fasting serum glucose, estimated GFR, serum bilirubin and spot urine ACR etc.) were measured by appropriate methods. We used student's t test and Pearson's correlation coefficient to detect the relationship of serum bilirubin with other variables. All statistical tests were considered at 5% level of significance. We found diabetic patients with nephropathy have significantly lower serum bilirubin ( $p < 0.001$ ) than diabetic patients without nephropathy ( $0.46 \pm 0.18$  and  $0.64 \pm 0.21$  mg/d respectively). Pearson's correlation test showed that serum bilirubin positively correlated with eGFR ( $r = 0.312$ ,  $p = 0.013$ ) in diabetic patients with nephropathy. This study concluded that low serum bilirubin level is associated with eGFR in diabetic patients with nephropathy.

**Key words:** Diabetes mellitus, Diabetic nephropathy, Bilirubin, eGFR, Spot urine ACR

### Introduction

The incidence of diabetes is increasing worldwide, with subsequent increase in the incidence of diabetic nephropathy<sup>1</sup>. In Bangladesh, there were 8.4 million adults living with diabetes in 2019, and projected to almost double (15.0 million) within the next twenty five years<sup>2</sup>. Diabetic nephropathy is the most

common cause of end-stage renal disease worldwide and accounts for a significant increase in mortality and morbidity in patients with diabetes<sup>3</sup>. Patients with diabetic nephropathy are diagnosed based on elevated urinary albumin excretion (UAE) and/or reduced estimated glomerular filtration rate (eGFR)<sup>4</sup>.

Oxidative stress plays a pivotal role in the development of microvascular complications, in diabetic patients including nephropathy<sup>5,6</sup>. Bilirubin, an endogenous product of heme catabolism, is a potent anti-oxidant that effectively scavenges peroxy radicals, and suppresses the oxidation of lipids and lipoproteins<sup>7</sup>.

The exact nature of the relationship of serum bilirubin level and development of nephropathy in diabetic patients is unknown. But it is expected that oxidative stress, the major pathogenic factor of diabetic nephropathy, can be reduced by increased level of serum bilirubin within physiological range<sup>6,8</sup>. Although several studies suggest a renoprotective role of serum bilirubin, others found that higher serum bilirubin levels may have a possible role in contributing to the development of renal failure, possibly linked to jaundice-related nephropathy<sup>9-12</sup>. But as far we know, there is no study regarding the relationship of serum bilirubin with eGFR and spot urine ACR in type 2 diabetic patients with or without nephropathy in Bangladesh. So this study aimed to find out any relation of serum bilirubin with estimated glomerular filtration rate (eGFR) and spot urine albumin to creatinine ratio in type 2 diabetic patients with or without nephropathy in our population.

### Materials and Methods

This cross sectional study was done in the department of Biochemistry and Molecular Biology, BIRDEM Academy from July 2017 to June 2018. In this study a total of 126 respondents, both male and female subjects between the age group of 30 to 60 years were selected from outpatient department of Medicine, BIRDEM General Hospital, among them 63 previously diagnosed type 2 diabetic patients

without nephropathy and 63 previously diagnosed type 2 diabetic patients with nephropathy. Patients with jaundice, acute kidney injury, kidney disease with non-diabetic etiology or patients on renal replacement therapy were excluded. Also pregnant women, patients having nephrotoxic or hepatotoxic drugs are excluded. A structured questionnaire was filled in for each respondent after taking informed written consent. Type 2 diabetic patients were previously diagnosed depending on history, clinical features and WHO criteria<sup>13</sup>. Nephropathy was diagnosed on the basis of persistent albuminuria ( $>30\text{mg/day}$  or  $\text{ACR} >30\text{mg/g}$ ) in at least two occasions within six months period and/ or GFR less than  $60\text{ ml/min/1.73m}^2$  for more than three months<sup>14</sup>. Serum creatinine was measured in Jaffe's method by Abbott ARCHITECT PLUS C 8000 auto analyzer. Estimated GFR was calculated by CKD-EPI method. Serum bilirubin was measured by photometric method in Abbott ARCHITECT PLUS C 8000 autoanalyzer. HbA1c was assessed in 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. Then urine microalbumin creatinine ratio (mg/g) was calculated. Hemoglobin was measured by Sodium lauryl sulphate method in SYSMEX XN-1000 autoanalyzer. Student's t-test and Pearson's correlation coefficient were done to determine the relation of serum bilirubin with spot urine ACR and eGFR. All statistical tests were considered at 5% level of significance at SPSS version 22.0. This study was approved by Institutional Review Board, BIRDEM.

## Results

In our study 126 diabetic patients aged 30 to 60 years were selected from outpatient department of Medicine from BIRDEM General Hospital according to inclusion criteria and divided into 2 groups based on presence and absence nephropathy, 63 patients of Type 2 DM without nephropathy, 63 patients of Type 2 DM with nephropathy.

**Table I:** Comparison of different characteristics between diabetic patients without nephropathy and diabetic patients with nephropathy

Variables	Diabetic patients without nephropathy (n=63)	Diabetic patients with nephropathy (n=63)	p values
	Mean ±SD	Mean ±SD	
Age (years)	48 ±9.18	52 ± 6.93	0.004
Duration of DM (years)	7.25±4.49	9.37±5.49	0.020
Body mass index (kg/m <sup>2</sup> )	25.7 ± 3.57	27.4 ± 4.11	0.015
Systolic blood pressure (mm of Hg)	123.57 ±14.60	129.92 ± 16.96	.036
Diastolic blood pressure (mm of Hg)	79.05±7.40	81.03 ± 7.47	0.137
Hemoglobin (g/dl)	13.95±1.26	12.84±1.58	<0.001
Fasting serum glucose (mmol/L)	9.07±2.94	10.17±4.33	0.099
HbA <sub>1c</sub> (%)	7.81±1.83	9.00±2.51	0.003
eGFR (ml/min/1.73m <sup>2</sup> )	78.14±14.51	40.63±13.07	<0.001
Urinary ACR (mg/g)	11.81±8.11	128.42±122.76	<0.001
Serum bilirubin (mg/dL)	0.64±0.21	0.46±0.18	<0.001

Table I showed the differences of clinical and biochemical variables among two groups (diabetic patients without nephropathy and diabetic patients with nephropathy). In this study it was found that age, duration of DM, BMI and systolic blood pressure were significantly higher in diabetic patients with nephropathy than diabetic patients without nephropathy.

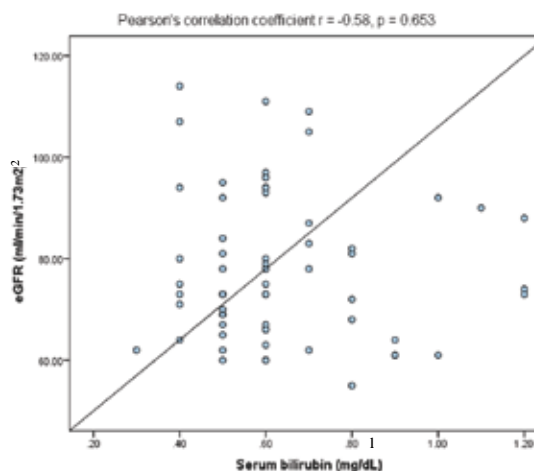
Mean hemoglobin, eGFR and serum bilirubin were significantly lower ( $p < 0.001$ ) in diabetic patients with nephropathy than other group. HbA<sub>1c</sub> ( $p < 0.01$ ) and urinary ACR ( $p < 0.001$ ) were significantly higher in diabetic patients with nephropathy than diabetic patients without nephropathy.

**Table II:** Correlation between serum bilirubin concentration and other variables in diabetic patients with or without nephropathy.

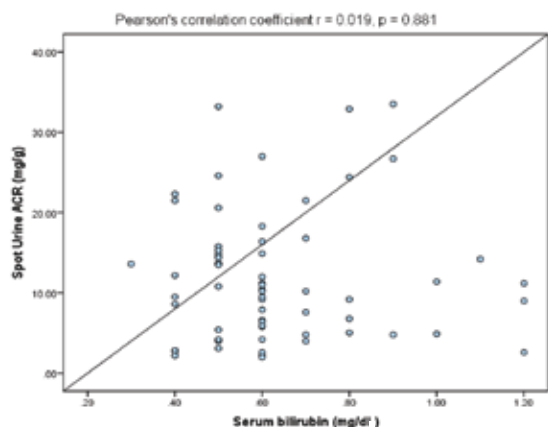
Variables	Diabetic patients without nephropathy (n=63)	Diabetic patients with nephropathy (n=63)
	r (p)	r (p)
Age (in years)	0.14 (0.914)	-0.059 (0.643)
Duration of DM (in years)	-0.035 (0.788)	-0.217 (0.088)
Body mass index (kg/m <sup>2</sup> )	0.161 (0.208)	0.064 (0.616)
Estimated GFR (ml/min/1.73m <sup>2</sup> )	-0.058 (0.653)	0.312 (0.013)
Spot Urine ACR (mg/g)	0.019 (0.881)	-0.051 (0.694)
Fasting serum glucose (mmol/L)	-0.112 (0.384)	-0.047 (0.715)

Statistical analysis was done by Pearson's correlation test. Values are expressed as the r: Pearson's correlation coefficient.

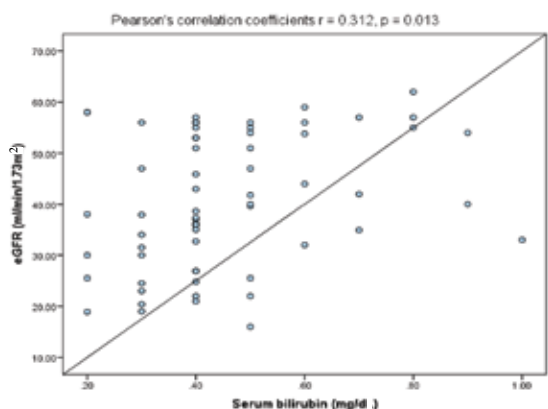
In this study we did not find any significant correlation of serum bilirubin with eGFR or spot urine ACR in diabetic patients without nephropathy. We found significant positive correlation of serum bilirubin with eGFR ( $r = 0.312$ ,  $p = 0.013$ ) in diabetic patients with nephropathy, but no significant correlation of serum bilirubin with spot urine ACR was found in this group.



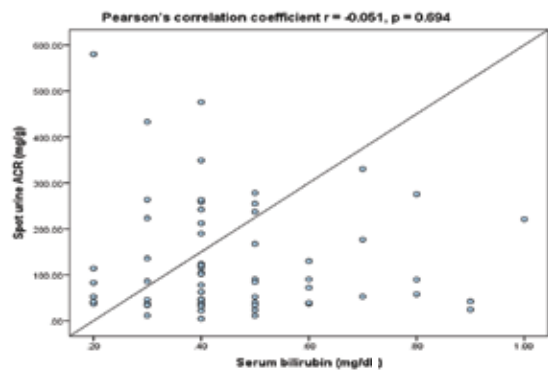
**Fig 1.** Relationship between serum bilirubin and estimated glomerular filtration rate (eGFR) in diabetic patients without nephropathy.



**Fig 2.** Relationship between serum bilirubin and spot urine albumin to creatinine ratio (ACR) in diabetic patients without nephropathy



**Fig 3.** Relationship between serum bilirubin and estimated glomerular filtration rate (eGFR) in diabetic patients with nephropathy.



**Fig 4.** Relationship between serum bilirubin and spot urine albumin to creatinine ratio (ACR) in diabetic patients with nephropathy

## Discussion

This study analyzed the relationship of serum bilirubin concentration with spot urine ACR and eGFR in type 2 diabetic patients with or without nephropathy. 126 diabetic patients were selected according to inclusion criteria (among them, 63 with nephropathy and 63 without nephropathy).

In current study 74.6% participants were male in diabetic patients without nephropathy. In contrast, 67.7% participants were male in diabetic patients with nephropathy. We found significantly higher ( $p=0.004$ ) mean age in diabetic patients with nephropathy ( $52 \pm 6.93$  years) than the other group ( $48 \pm 9.18$  years). BMI was also found significantly higher ( $p < 0.05$ ) in diabetic patients with nephropathy in comparison with diabetic patients without nephropathy ( $27.4 \pm 4.11 \text{ kg/m}^2$  and  $25.7 \pm 3.57 \text{ kg/m}^2$  respectively). Both these findings were similar with other studies<sup>15-17</sup>.

In present study, mean systolic blood pressure in diabetic patients with or without nephropathy were  $129.92 \pm 16.96$  mm of Hg and  $123.57 \pm 14.60$  mm of Hg respectively; and mean diastolic blood pressure were  $81.03 \pm 7.47$  mm of Hg and  $79.05 \pm 7.40$  mm of Hg respectively. Mean systolic blood pressure was significantly higher ( $p=0.036$ ) in diabetic patients with nephropathy than other group which reflects the finding of some other studies<sup>15,16</sup>.

Mean hemoglobin was significantly lower ( $p < 0.001$ ) in diabetic patients with nephropathy than diabetic patients without nephropathy ( $12.84 \pm 1.58 \text{ g/dl}$ ,  $13.95 \pm 1.26 \text{ g/d}$  respectively). Fan et al also found mean hemoglobin was significantly lower ( $p < 0.01$ ) in diabetic nephropathy patients than healthy control and diabetic patients ( $117 \pm 27 \text{ g/L}$ ,  $149 \pm 20 \text{ g/L}$ ,  $140 \pm 14 \text{ g/L}$  respectively)<sup>15</sup>.

In this study we did not find any significant difference of fasting serum glucose in diabetic patients with or without nephropathy. Mean

HbA1c was found significantly higher ( $9.00 \pm 2.51\%$ ) in diabetic patients with nephropathy than diabetic patient without nephropathy ( $7.81 \pm 1.83\%$ ). Both these findings were consistent with the other studies<sup>15,16</sup>.

In addition, the present study found that eGFR was significantly lower ( $p < 0.001$ ) diabetic patients with nephropathy than diabetic patient without nephropathy ( $40.63 \pm 13.07 \text{ ml/min/1.73m}^2$  and  $78.14 \pm 14.51 \text{ ml/min/1.73m}^2$  respectively). Mean serum bilirubin in diabetic patients with and without nephropathy were  $0.46 \pm 0.19 \text{ mg/dL}$  and  $0.64 \pm 0.21 \text{ mg/dL}$  respectively. Serum bilirubin were significantly lower in diabetic patients with nephropathy ( $p < 0.001$ ) than diabetic patients without nephropathy. Similar findings were reported in other studies<sup>15-17</sup>.

In this study, we found no significant correlation of serum bilirubin with spot urine ACR ( $r = 0.019$ ,  $p = 0.881$ ) and eGFR ( $r = -0.058$ ,  $p = 0.653$ ) in diabetic patients without nephropathy. Katoh et al found significant positive correlation of serum bilirubin with eGFR in diabetic patients<sup>9</sup>. The reason behind the different results may be due to different ethnicity of population and smaller sample size in our study.

In current study, serum bilirubin is positively correlated with eGFR ( $r = 0.312$ ,  $p = 0.013$ ) in diabetic patients with nephropathy. But we did not find any significant correlation of serum bilirubin with spot urine ACR in this group. Fukui et al found serum bilirubin is positively correlated with estimated glomerular filtration rate ( $r = 0.106$ ,  $p = 0.0265$ ) and negatively correlated with logarithmic (log) urinary albumin excretion ( $r = -0.202$ ,  $p = 0.0001$ )<sup>10</sup>. Many other studies found positive association of serum bilirubin with eGFR and negative association of serum bilirubin with albuminuria<sup>9-11,18</sup>. The possible reason for these differences may be due to differences in study design and the sample size of the study participant.

### **Limitation of the study**

This study was done in a limited period of time with relatively small population and convenient sampling was used from a single center. We did not differentiate direct and indirect bilirubin from total serum bilirubin. Multicenter, longitudinal, population based study with a large sample size and longer duration is recommended for more accurate and reliable results.

### **Conclusion**

In this study, significant lower serum bilirubin level was observed in diabetic patients with nephropathy in comparison with diabetic patients without nephropathy. We found serum bilirubin was positively correlated with eGFR in diabetic patients. Our results suggest that low serum bilirubin level may predict the risk of development and progression of nephropathy in diabetic patients with nephropathy. Serum bilirubin is a simple, cost effective biochemical test. So, this study concluded that routine screening of serum bilirubin in type 2 diabetic patients may be beneficial for early diagnosis, prevention of progression and early treatment of nephropathy in type 2 diabetic patients.

### **Institutional Review Board Statement**

This study was approved by Institutional Review Board, BIRDEM

### **Informed Consent Statement**

Informed written consent was taken from each respondent.

### **Conflicts of Interest**

The author declares no conflict of interest.

### **Acknowledgement**

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