

Relationship between different normal serum bilirubin concentrations and diabetic nephropathy in patients with type 2 diabetes mellitus

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ABSTRACT

Background: One of the major complications of type 2 diabetes is diabetic nephropathy (DN). Nephropathy develops in ~20–40% of patients with diabetes and is the single leading cause of end-stage renal disease (ESRD) around the world.(1) Oxidative stress has been considered to be an important pathogenic factor in the development of diabetic vascular complications, including nephropathy. Bilirubin(BIL) has been recognized as an endogenous antioxidant.

Objective: The objective of this study is to detect the relationship between serum bilirubin concentrations and diabetic nephropathy in patients with type 2 diabetes mellitus.

PATIENTS & METHODS:The study included 92 patient with type 2 diabetes mellitus who visit Sohag University Hospital ,All patients in this study were subjected to full history taking, complete physical examination, and laboratory investigations including urine analysis, albumin creatinine ratio, renal and liver function tests. Patients with T2DM were recruited and bilirubin concentrations were compared between patients with or without diabetic nephropathy. DN was diagnosed according to KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease⁽⁷⁾

Patients were classified according to stage of nephropathy⁽⁸⁾:

- 1-stage 1 ----- albumin creatinine ratio less than 30 mg/g creatinin.
- 2-stage 2 ----- morphologic lesions without signs of clinical disease.
- 3-stage 3 ----- albumin creatinine ratio more than 30 , less than 300 mg/g creatinin.
- 4-stage 4 ----- albumin creatinine ratio more than 300 mg/g creatinin.
- 5- stage 5 ----- end stage renal disease.

Results: Multiple stepwise regression analysis was used to examine the relationship between bilirubin concentrations (within normal range) and albumin creatinin ratio.

Conclusions

Serum bilirubin concentration correlated negatively with stage of diabetic nephropathy, which suggests that bilirubin has a potential role for protection of diabetic nephropathy.

Introduction

Nephropathy develops in ~20–40% of patients with diabetes and is the single leading cause of end-stage renal disease (ESRD) around the world.(1)

In recent years, oxidative stress has been considered to be an important pathogenic factor in the development of diabetic vascular complications, including nephropathy.

Bilirubin(BIL) has been recognized as an endogenous antioxidant for many years,as its formation is mediated by the expressed hemeoxygenase (HO), the rate-limiting enzyme involved in heme catabolism.(2)

HO participates in heme breakdown to generate biliverdin(BVD), free ferrous iron and carbon monoxide. Subsequently, BVD is rapidly converted to BIL by BVD reductase. Recently, increasing evidence has suggested that HO and its reaction product BIL may serve as important endogenous agents with cytoprotective activity against oxidative stress injury.(3-5)

Information on the associations of serum bilirubin concentration with renal function and proteinuria is limited and controversial. studies found that total serum bilirubin was positively associated with eGFR and negatively associated with albuminuria in a hospital-based sample of 633 Japanese type 2 diabetic patients(6), indicating that bilirubin has a potential renoprotective effect .

Other study reviewed the medical records of 1363 adults aged 18 years or older who were seen at Kosin University Gospel Hospital (Busan, Republic of Korea) in the five-year period from January 2005 to December 2009. This study demonstrated that serum total bilirubin concentration was negatively correlated with 24-hour urine protein and was positively correlated with eGFR in Korean non-diabetic and diabetic adults.

PATIENTS & METHODS:

-Place of the study: Sohag University Hospital

-Type of the study: Cross sectional clinical based study.

-Study period: 6 months

- Patients:

Inclusion criteria:

All type 2 diabetic patients.

All patients with serum bilirubin concentrations within normal range.

All stage of diabetic nephropathy except stage 5.

Exclusion criteria:

Type 1 diabetic patients.

Patients with urinary tract infection.

Patients on regular dialysis.

Pregnancy.

Patients with any conditions that can affect bilirubin concentrations as any liver disease, blood diseases, malignancy.

Methods :

All patients in this study were subjected to full history taking, complete physical examination, and laboratory investigations including urine analysis, albumin creatinine ratio, renal and liver function tests. Patients with T2DM were recruited and bilirubin concentrations were compared between patients with or without diabetic nephropathy. DN was diagnosed according to KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease .(7) Patients were classified according

to stage of nephropathy (8):

1-stage 1 ----- albumin creatinine ratio less than 30 mg/g creatinin.

2-stage 2 ----- morphologic lesions without signs of clinical disease.

3-stage 3 ----- albumin creatinine ratio more than 30 , less than 300 mg/g creatinin.

4-stage 4 ----- albumin creatinine ratio more than 300 mg/g creatinin.

5- stage 5 ----- end stage renal disease.

Statistical analysis:

Multiple stepwise regression analysis was used to examine the relationship between bilirubin concentrations and albumin creatinin ratio. Data was analyzed using STATA intercooled version 14.2. Quantitative data was represented as mean, standard deviation, median and range. Data was analyzed using student t-test to

compare means of two. When the data was not normally distributed Mann-Whitney test was used to compare two groups. Test for trend was used to compare quantitative data in ordered group. Qualitative data was presented as number and percentage and compared using either Chi square test

or fisher exact test. Pearson's correlation analysis was used find the correlation between different variables. Univariate and multivariate regression analysis was used to find factors affecting albumin creatinine ration. Graphs were produced by using Excel or STATA program.

Results:

Initially the study included 163 patients with type 2 diabetes mellitus who visit Sohag University Hospital in a period of 6 months from 1/12/2017 to 1/5/ 2018. 71 patients were excluded from the study and 92 patients finally included and were analyzed. The mean age of the study group was 53 years (SD: 7.7 years). Range was 38-72 years. Regarding sex, 43 patients were males (47.25%) and 48 patients were females (52.7%). (Table 1).

(Table 1): Age and gender distribution of studied population

Variable	Summary statistics
Age/year	
Mean ± SD	53.24±7.76
Median (range)	51 (38-72)
Gender	
Females	43 (47.25%)
Males	48 (52.75%)

Regarding medical history of the studied population, as regard smoking, 49.45% never smoke (45 patients) while 20.88 % of our study group had past history of smoking (19 patients). And 27 patients of our study group are current smoker (29.7%) (Table 2). Regarding the hypertension, 47 patients were not hypertensive in our study group (51.65%) while 44 patients were hypertensive (48.35%) (Table 2). Regarding the duration of diabetes, the mean 9.87 with (SD: 6.35), range was (1- 31) years (table 2). As regard type of diabetic treatment, (51.65%) of the patients were on oral treatment (47) patients, while 44 patients (48.35%) were on insulin treatment (table 2).

(Table 2): Medical history of studied population

Variable	Summary statistics
Smoking	
Never	45 (49.45%)
Past	19 (20.88%)
Current	27 (29.67%)
Hypertension	
No	47 (51.65%)
Yes	44 (48.35%)
Duration of diabetes	
Mean ± SD	9.87±6.35
Median (range)	9 (1-31)
Type of diabetic treatment	
Oral	47 (51.65%)
Insulin	44 (48.35%)

Regarding the stage of diabetic nephropathy, 35 patients of the study group were stage 1 and 2(38.46%). 36 patients were stage 3 (39.56%), and 20 patients were stage 4(21.98%). (Table 3)

(Table 3) Stage of diabetic nephropathy of studied population

Kidney disease stage	Summary statistics
Stage 1 & 2	35 (38.46%)
Stage 3	36 (39.56%)
Stage 4	20 (21.98%)

Regarding the laboratory investigations of the study population, the mean of total bilirubin 0.76 (SD: 0.27; range: 0.2-1.2) (Table 4). The mean of serum creatinine was 1.03 (SD: 0.34; range: 0.3-2.1), (table 4). Regarding Albumin creatinine ratio, the mean was 283.21 (SD: 460.69; range: 1-2102). (Table 4)

(Table 4) Lab investigations of studied population

Variable	Summary statistics
Total bilirubin Mean ± SD Median (range)	0.76±0.27 0.8 (0.2-1.2)
Serum creatinine Mean ± SD Median (range)	1.03±0.34 1 (0.3-2.1)
Albumin creatinine ratio Mean ± SD Median (range)	283.21±460.69 52 (1-2102)

Multiple analysis were done to find any relationship between total bilirubin and different variables of the study (gender, smoking, hypertension, type of diabetic treatment and stage of diabetic nephropathy). (Table 5)

(Table5) Relationship of total bilirubin and different variables

Variable	Number	Mean ± SD, median (range)	P value
Gender			
Females	43	0.72±0.23, 0.7 (0.3-1.2)	0.30
Males	48	0.79±3.1, 0.85 (0.2-1.2)	
Smoking			
Never	45	0.73±0.24, 0.8 (0.2-1.2)	0.31
Past	19	0.77±0.34, 0.8 (0.3-1.2)	
Current	27	0.8±0.26, (0.8-0.2)	
Hypertension			
No	47	0.79±0.25, 0.8 (0.3-1.2)	0.21
Yes	44	0.72±0.29, 0.8 (0.2-1.2)	
Type of diabetic treatment			
Oral	47	0.77±0.25, 0.8(0.2-1.2)	0.53
Insulin	44	0.74±0.29, 0.8(0.2-1.2)	
Kidney disease stages			
Stage 1 & 2	35	0.96±0.20, 1 (0.3-1.2)	<0.0001
Stage 3	36	0.73±0.17, 0.7 (0.4-1.1)	
Stage 4	20	0.45±0.19, 0.4 (0.2-1.0)	

Our results show that there was no relationship between total bilirubin and different variables (gender, smoking, hypertension and type of diabetic treatment). While there was obvious relationship between total bilirubin and stage of diabetic nephropathy (p value <0.0001)

Multiple analysis were done to find relationship of serum creatinin and different variables of the study (gender, smoking, hypertension, type of diabetic treatment and stage of diabetic nephropathy). (Table 6)

(Table6) Relationship of serum creatinine and different variables

Variable	Number	Mean ± SD, median (range)	P value
Gender			
Females	43	0.98±0.31, 1.0 (0.4-1.9)	0.18
Males	48	1.08±0.37, 1.0 (0.3-2.1)	
Smoking			
Never	45	0.98±0.32, 0.9 (0.4-1.9)	0.56
Past	19	1.08±0.29, 1.0 (0.7-1.9)	
Current	27	1.07±0.42, 1.0 (0.3-2.1)	
Hypertension			
No	47	0.96±0.30, 0.9 (0.4-1.9)	0.06
Yes	44	1.1±0.37, 1.0 (0.3-2.1)	
Type of diabetic treatment			
Oral	47	0.99±0.31, 1.0 (0.3-1.9)	0.38
Insulin	44	1.06±0.38, 1.0 (0.4-2.1)	
Kidney disease stage			
Stage 1 & 2	35	0.84±0.28, 0.8 (0.3-1.8)	<0.0001
Stage 3	36	1.03±0.26, 1.0 (0.4-1.9)	
Stage 4	20	1.36±0.35, 1.3 (0.8-2.1)	

Our results show that there was no relationship between serum creatinin and different variables (gender, smoking, hypertention and type of diabetic treatment). While there was obvious relationship between it and stage of diabetic nephropathy (p value <0.0001).

Multiple analyses were done to find relationship of albumin creatinine ratio and different variables of the study (gender, smoking, and hypertension, type of diabetic treatment and stage of diabetic nephropathy). (Table7)

(Table7) Relationship of albumin creatinine ratio and different variables

Variable	Number	Mean ± SD, median (range)	P value
Gender			
Females	43	230.51±301.22, 111 (2-1302)	0.38
Males	48	330.42±566.15, 42(1-2102)	
Smoking			
Never	45	267.31±407.70, 111 (2-2102)	0.37
Past	19	294.89±442.53, 92 (3-1313)	
Current	27	301.48±562.97, 40 (1-2100)	
Hypertension			
No	47	180.21±279.47, 49 (1-1302)	0.22
Yes	44	393.23±580.25, 94.5 (2-2102)	
Type of diabetic treatment			
Oral	47	235.47±406.29, 50 (2-2102)	0.66
Insulin	44	334.20±512.34, 66.5 (1-2100)	
Diabetic nephropathy stage			
Stage 1 & 2	35	18.37±10.14, 23 (1-30)	<0.0001
Stage 3	36	144.22±95.74, 125.5 (31-321)	
Stage 4	20	966.85±535.16, 856 (361-2102)	

Our results show that there was no relationship between serum creatinin and different variables (gender, smoking, hypertension and type of diabetic treatment). While there was obvious relationship between it and stage of diabetic nephropathy (p value <0.0001).

There was correlation between total bilirubin and duration of diabetes (p value0.01) , serum creatinine (p value .001) and albumin creatinie ratio (p value <0.0001) (Table 8)

(Table 8) Correlation between total bilirubin and different quantities variable

Variable	Correlation co-efficient	P value
Age/year	-0.21	0.04
Duration of diabetes	-0.26	0.01
Serum creatinine	-0.35	0.001
Albumin creatinine ratio	-0.72	<0.0001

There was correlation between serum creatinine and duration of diabetes (p value 0.01), serum bilirubin (p value .001) and albumin creatinine ratio (p value <0.0001) (Table 9).

(Table 9) Correlation between serum creatinine and different quantities variable

Variable	Correlation co-efficient	P value
Age/year	0.27	0.01
Duration of diabetes	0.29	0.01
Total bilirubin	-0.36	0.001
Albumin creatinine ratio	0.54	<0.0001

There was correlation between Albumin creatinine ratio and duration of diabetes (p value 0.001), serum bilirubin (p value <0.0001) and serum creatinine (p value <0.0001)

(Table 10) Correlation between Albumin creatinine ratio and different quantities variable

Variable	Correlation co-efficient	P value
Age/year	0.21	0.04
Duration of diabetes	0.34	0.001
Total bilirubin	-0.72	<0.0001
Serum creatinine	0.54	<0.0001

Our results also showed that there was a significant negative relationship between total bilirubin and albumin creatinine ratio.

(Table 11) Univariate regression analysis of factors affecting albumin creatinine ratio

Variable	Regression coefficient (95% CI)	P value
Age/years	12.44 (0.22-24.67)	0.046
Male gender	99.91 (-92.23:292.04)	0.30
Past smoker vs. past	27.58 (-225.58:280.75)	0.83
Past smoker vs. current	34.17 (-191.08:259.43)	0.76
Hypertension	213.01 (25.20-400.83)	0.03
Duration of diabetes	25.20 (10.88-39.53)	0.001
Insulin Vs. oral	98.74 (-93.24:290.71)	0.31
Total bilirubin	-1220.32 (-1469.86:-970.77)	<0.0001

(Table 12) Multivariate regression analysis of factors affecting albumin creatinine ratio

Variable	Regression coefficient (95% CI)	P value
Age/years	-2.57 (-13.02:7.89)	0.63
Male gender	229.72 (-88.33:467.76)	0.36
Past smoker vs. past	-142.49 (-406.64:121.66)	0.29
Past smoker vs. current	-70.97 (-315.15:173.21)	0.57
Hypertension	63.94 (-75.40:203.28)	0.36
Duration of diabetes	13.21 (0.15:26.29)	0.048
Insulin Vs. oral	21.48 (-112.84:155.79)	0.75
Total bilirubin	-1166.80 (-1420:-912.88)	<0.0001

At the end our result we found that albumin creatinine ratio can be affected by both duration of diabetes and total bilirubin (Table 11,12 and 13)

(Table 13) Final factors affecting albumin creatinine ratio

Variable	Regression coefficient (95% CI)	P value
Duration of diabetes	11.49 (1.01:21.96)	0.03
Total bilirubin	-1183.75 (-1430.82:-936.68)	<0.0001

Discussion

Our study assessed the relationship between different normal serum bilirubin concentrations and diabetic nephropathy in patients with type 2 diabetes mellitus.

Initially the study included 163 patients with type 2 diabetes mellitus who visit Sohag University Hospital in a period of 6 months from 1/12/2017 to 1/5/ 2018. 71 patients were excluded from the study and 92 patients finally included and were analyzed.

The mean of the age of the study group was 53 years, 43 patients were males and 48 patients were females and this was not significant statistically.

Our study demonstrated that serum total bilirubin concentration was negatively correlated to albumin creatinine ratio which ensures the role of bilirubin as antioxidant giving renoprotective effect.

As regard relationship between total bilirubin and different variables of the study (gender, smoking, hypertension, type of diabetic treatment and stage of diabetic nephropathy). Our results show that there was no relationship between total bilirubin and different variables (gender, smoking, hypertension and type of diabetic treatment). While there was obvious relationship between total bilirubin and stage of diabetic nephropathy (p value <0.0001).

In agreement with our results, Fukui et al.⁽⁹⁾ That previous study found that total serum bilirubin was positively associated with eGFR and negatively associated with albuminuria in a hospital-based sample of 633 Japanese type 2 diabetic patients (mean age: 64.4 ± 11.5 years; 52% male).

Another study Ho Sik Shin, Yeon Soon Jung, and Hark Rim⁽¹⁰⁾, inverse correlation was found between total serum bilirubin concentration and 24-hour urine albumin in all type 2 diabetes mellitus

In contrast to our study, the results of Targher et al⁽¹¹⁾ in their observational large hospital-based sample of 2678 adult outpatients (mean age: 55 ± 18 years; 43% male), including 210 diabetic patients. In that study, they found that serum total bilirubin was inversely associated with eGFR in both non-diabetic (r = -0.17; p < 0.0001) and diabetic patients (r = -0.14; p < 0.05). However, no information was available on albuminuria,

As regard stage of diabetic nephropathy (p value <0.0001) correlate negatively with the serum total bilirubin at our study. In agreement with our results Ho Sik Shin, Yeon Soon Jung, and Hark Rim, Serum bilirubin concentrations were lower in patients with macroalbuminuria than in patients with normoalbuminuria (P<0.0001) or microalbuminuria (P=0.0012), and serum bilirubin concentrations were lower in patients with microalbuminuria than in patients with normoalbuminuria (P=0.0418).

During our study, multiple analyses were done to find relationship of albumin creatinine ratio and different variables of the study (gender, smoking, and hypertension, type of diabetic treatment and stage of diabetic nephropathy). Our results show that there was no relationship between total bilirubin and different variables (gender, smoking, hypertension and type of diabetic treatment). While there

was obvious relationship between total bilirubin and stage of diabetic nephropathy, and our result also found that albumin creatinine ratio can be affected by both duration of diabetes and total bilirubin. But Fukui et al⁽⁹⁾ showed multiple regression analysis demonstrated that age, duration of diabetes, hemoglobin A1C (HbA1c), systolic blood pressure, serum triglyceride concentration, and serum bilirubin concentration were independent determinants of log (*urinary albumin excretion*)

In agreement to our study Fukui et al⁽⁹⁾ showed that serum bilirubin concentrations did not differ between patients treated with and without insulin ($P=0.3863$)

In contrast to our study, Fukui et al⁽⁹⁾ showed that Serum bilirubin concentrations were lower in patients with current smokers than in patients with past smokers ($P=0.0294$). But, our study show no relationship between serum bilirubin concentrations and smoking ($P=0.31$)

Our results found that (48.53%) of the patients were hypertensive and diabetic and (Ho Sik Shin, Yeon Soon Jung⁽¹⁰⁾ found that Diabetes and hypertension were present in 44.9% of patients

Recommendation: We recommend to perform usual testing for total bilirubin in patients with type 2 diabetes mellitus which can be indicator for stage of nephroathy .

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