

Association between serum bilirubin and albuminuria in type 2 diabetes mellitus and diabetic nephropathy

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Abstract

Introduction: Diabetic nephropathy develops due to oxidative stress and inflammation resulting from chronic hyperglycemia. Bilirubin, a product of heme catabolism is found to have antioxidant and anti-inflammatory properties. Though previous studies have examined the relationship between total bilirubin and diabetic nephropathy, very few studies have focused on indirect and direct bilirubin levels. Hence, the present study aimed to compare serum bilirubin (total, indirect and direct) levels between non-diabetics, type 2 diabetics and diabetic nephropathy subjects and also to correlate albuminuria with serum bilirubin in type 2 diabetics and diabetic nephropathy subjects.

Materials and Methods: 50 non-diabetics, 50 type 2 diabetics and 50 diabetic nephropathy subjects were included in the study. Fasting blood glucose, HbA_{1c}, serum bilirubin (total, indirect and direct), serum creatinine, urine microalbumin and urine creatinine were measured. Estimated glomerular filtration rate (eGFR) and urine albumin creatinine ratio (ACR) was calculated.

Results: Total bilirubin, direct and indirect bilirubin were significantly decreased in type 2 diabetics and diabetic nephropathy subjects compared to non-diabetics. Total bilirubin and indirect bilirubin were also significantly decreased in diabetic nephropathy subjects compared to type 2 diabetics. Total bilirubin, direct and indirect bilirubin showed significant negative correlation with albuminuria (Urine ACR) in type 2 diabetics and diabetic nephropathy subjects.

Conclusion: Our study suggests that low bilirubin levels might be a risk factor for the development of type 2 diabetes and diabetic nephropathy. Bilirubin can be used as a marker to predict the risk of developing type 2 diabetes among general population and diabetic nephropathy among type 2 diabetes patients.

Keywords: Albuminuria, Antioxidant, Bilirubin, Diabetes mellitus, Diabetic nephropathy.

Introduction

Diabetic nephropathy is one of the major complications of type 2 diabetes mellitus. Nephropathy occurs in about 20-40% of patients with type 2 diabetes mellitus and it is the leading cause of end stage renal disease around the world.¹⁻² Diabetic nephropathy develops due to oxidative stress and inflammation resulting from chronic hyperglycemia.²⁻³ Diabetic nephropathy is diagnosed by elevated urinary albumin creatinine ratio (albuminuria) and/or reduced estimated glomerular filtration rate.⁴ The only option available to prevent this complication is strict glycemic control. But nephropathy is also seen to occur in patients under strict glycemic control.³ So there is a need to identify a novel biomarker that can predict the risk for diabetic nephropathy.

Bilirubin is a product of heme catabolism by heme oxygenase and it is excreted by liver cells.⁵ Previously, bilirubin is thought to be a toxic waste product,⁶ but recent studies have reported that bilirubin has antioxidant, anti-inflammatory and antiapoptotic properties.² Both direct and indirect bilirubin are found to have these properties.⁷ Previous studies have found that serum total bilirubin levels are inversely associated with the risk of diabetic nephropathy. Higher total

bilirubin levels were found to be protective against diabetic nephropathy.²

Though there are studies that focus on total bilirubin levels, very few studies have focused on indirect and direct bilirubin levels² and the studies are also lacking in Indian population. Hence, the present study aimed to compare serum bilirubin (total, indirect and direct) levels between non-diabetics, type 2 diabetics and diabetic nephropathy subjects and also to study the correlation of albuminuria with serum bilirubin levels in type 2 diabetics and diabetic nephropathy subjects.

Materials and Methods

The study was a hospital based cross-sectional study conducted in the Department of Biochemistry, Aarupadai Veedu Medical College & Hospital, Puducherry, India. The study was approved by our Institutional Research Committee and Institute Ethics Committee (human studies). The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

One hundred and fifty subjects aged 30 years and above were recruited from the department of Medicine & Diabetology (both outpatients and inpatients) at

Aarupadai Veedu Medical College & Hospital, Puducherry. The study subjects were divided into three groups: non-diabetics (age and sex matched fifty healthy subjects), type 2 diabetics (n=50) and diabetic nephropathy (n=50). Patients with liver diseases, hemolytic diseases, hypertension, cardiovascular disease and nondiabetic chronic kidney disease were excluded from the study.

After obtaining written informed consent from the study subjects, a detailed clinical history was collected from them through a pre-tested, semi-structured questionnaire. Height and weight were measured from all the study subjects and BMI was calculated by the formula: weight in kg/ height in m². Blood pressure was measured twice in right arm in sitting position using standard mercury sphygmomanometer (after 10 min rest).

5mL of fasting venous blood sample and urine samples were collected from all the study subjects. Fasting blood glucose, HbA_{1c}, serum bilirubin (total, indirect and direct), serum creatinine, urine microalbumin and urine creatinine were assayed in the fully automated biochemistry analyzer (Mindray BS-380). eGFR was calculated using MDRD equation $eGFR = 186.3 \times (\text{plasma creatinine})^{-1.154} \times \text{age}^{-0.203}$ ($\times 0.742$ for women). Urine albumin creatinine ratio (ACR) was calculated using the values of urine microalbumin and urine creatinine.

Statistical analysis

Normally distributed variables are expressed as mean with standard deviation and non-normally distributed variables are expressed as median (interquartile range). Comparison of anthropometric variables and biochemical parameters among non-diabetics, type 2 diabetics and diabetic nephropathy subjects were assessed by one-way ANOVA (for

normal data) and Kruskal-Wallis test (for non-normal data). Correlation analysis of albuminuria (Urine ACR) with total, direct and indirect bilirubin was done using Spearman Rank correlation. A p value of <0.05 was considered as statistically significant. All the statistical analysis were performed by using SPSS software version 16 (SPSS Inc., USA).

Results

A total of 150 subjects were included in the study. Characteristics of study population are shown in Table 1. No significant difference was found in age, height, weight, body mass index, systolic and diastolic blood pressure among non-diabetics, type 2 diabetics and diabetic nephropathy subjects.

Fasting blood glucose and HbA_{1c} were significantly increased in type 2 diabetics and diabetic nephropathy subjects compared to non-diabetics. Diabetic nephropathy subjects had significantly increased urine ACR levels compared to non-diabetics and type 2 diabetics. Total bilirubin, direct and indirect bilirubin were significantly decreased in type 2 diabetics and diabetic nephropathy subjects compared to non-diabetics. Total bilirubin and indirect bilirubin were also significantly decreased in diabetic nephropathy subjects compared to type 2 diabetics (Table 2). Comparison of total, direct and indirect bilirubin levels between non-diabetics, type 2 diabetics and diabetic nephropathy subjects is shown in Fig. 1.

Total bilirubin, direct and indirect bilirubin showed significant negative correlation with albuminuria (urine ACR) in type 2 diabetics and diabetic nephropathy subjects. Correlation analysis between bilirubin and albuminuria is shown in Table 3, Fig. 2 and Fig. 3.

Table 1: Characteristics of study population

Variables	Non diabetics (n=50)	Type 2 diabetics (n=50)	Diabetic nephropathy (n=50)
Age (years)	50.9±8.8	53.7±14.6	56.7±14.3
Height (cm)	162.5±7.9	162.3±7.7	163.2±7.6
Weight (kg)	67.3±10.8	68.8±8.9	66.6±9.4
Body mass index (kg/m ²)	25.4±3.2	26.1±2.5	25±3.2
Systolic blood pressure (mm Hg)	117±8.1	117.5±10.2	116.4±6.9
Diastolic blood pressure (mm Hg)	75±6.7	75.8±6.0	74.4±6.1

Data are expressed as mean±SD

Table 2: Comparison of biochemical parameters between non-diabetics, type 2 diabetics and diabetic nephropathy subjects

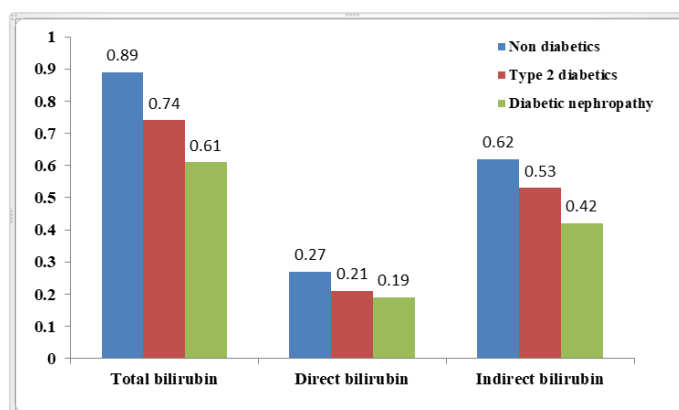
Variables	Non diabetics (n=50)	Type 2 diabetics (n=50)	Diabetic nephropathy (n=50)
Fasting blood glucose (mg/dL)	77.4±9.3	158.8±67.5*	168.6±81.2*
HbA _{1c} (%)	5.3±0.8	7.6±1.4*	7.9±1.6*
Creatinine (mg/dL)	0.72±0.13	0.81±0.21	0.89±0.58
eGFR (mL/min/1.73m ²)	115.9±30.3	105.4±41.6	103.5±42.3
Urine ACR (mg/g of cr)	20.5 (10)	23.5 (10)	68 (81)*#
Total Bilirubin (mg/dL)	0.89±0.13	0.74±0.29*	0.61±0.29*#
Direct Bilirubin (mg/dL)	0.27±0.06	0.21±0.08*	0.19±0.07*
Indirect Bilirubin (mg/dL)	0.62±0.12	0.53±0.24*	0.42±0.25*#

eGFR – estimated glomerular filtration rate; ACR – albumin creatinine ratio; Data are expressed as mean±SD; Urine ACR expressed as median (interquartile range); *p<0.05 compared to non diabetics; #p<0.05 compared to type 2 diabetics

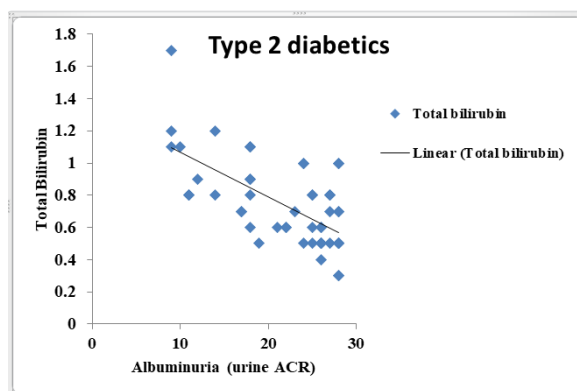
Table 3: Correlation of bilirubin with albuminuria (urine ACR) in type 2 diabetics and diabetic nephropathy subjects

Variables	Type 2 diabetics		Diabetic nephropathy	
	r value	p value	r value	p value
Total Bilirubin	-0.654	<0.001*	-0.509	<0.001*
Direct Bilirubin	-0.498	0.002*	-0.353	0.012*
Indirect Bilirubin	-0.603	<0.001*	-0.558	<0.001*

*p value< 0.05, statistically significant

**Fig.1: Comparison of total, direct and indirect bilirubin between non diabetics, type 2 diabetics and diabetic nephropathy subjects**

(A)



(B)

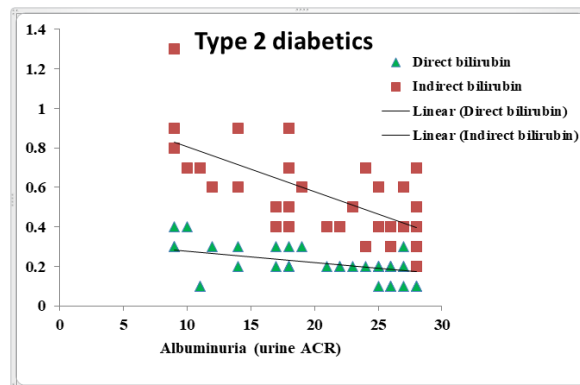
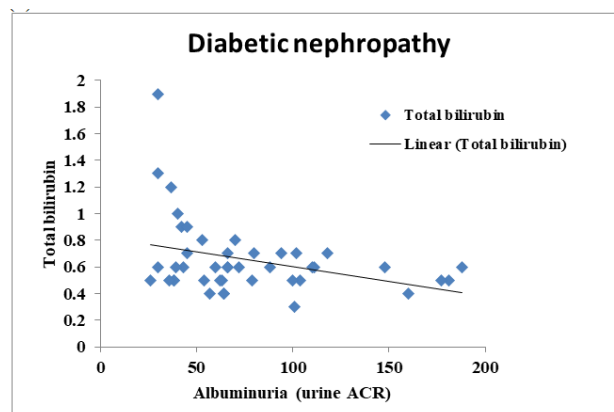


Fig. 2: (A) Correlation between total bilirubin and albuminuria (urine ACR) in type 2 diabetics, (B) Correlation between direct and indirect bilirubin and albuminuria (Urine ACR) in type 2 diabetics

(A)



(B)

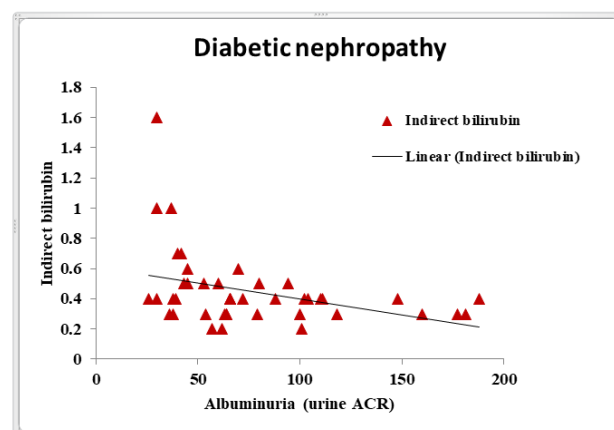


Fig. 3: (A) Correlation between total bilirubin and albuminuria (urine ACR) in diabetic nephropathy subjects, (B) Correlation between indirect bilirubin and albuminuria (Urine ACR) in diabetic nephropathy subjects

Discussion

This study was done to compare serum bilirubin (total, indirect and direct) levels between non-diabetics, type 2 diabetics and diabetic nephropathy subjects and also to correlate albuminuria (urine ACR) with serum

bilirubin levels in type 2 diabetics and diabetic nephropathy subjects.

In this study, we found that total bilirubin and its sub types, both direct and indirect bilirubin were significantly decreased in type 2 diabetics, compared to non-diabetics. This suggests that higher bilirubin might prevent the development of type 2 diabetes. Similar

findings were observed in a study conducted in Korean men, where they found a negative association between elevated serum bilirubin level and development of type 2 diabetes.⁸ Inverse correlation was also seen between bilirubin and the prevalence of type 2 diabetes mellitus in Korean men and women.⁶

A state of chronic hyperglycemia, seen in type 2 diabetes leads to the increased production of free radicals from mitochondria. This occurs most commonly in vascular endothelial cells, resulting in vascular dysfunction, a characteristic feature seen in type 2 diabetes as well as in diabetic complications.⁵ Bilirubin, being a potent anti-oxidant, might compensate this oxidative stress and prevent the development of type 2 diabetes.⁹

On comparing bilirubin levels between type 2 diabetics and diabetic nephropathy, we found that total bilirubin and indirect bilirubin, but not direct bilirubin, were significantly decreased in diabetic nephropathy subjects compared to type 2 diabetics. Though, total bilirubin includes the sum of direct and indirect bilirubin, indirect bilirubin accounts for 96% of total bilirubin. So, in spite of the fact that both direct and indirect bilirubin have beneficial properties,⁷ in our study, only indirect bilirubin was significantly decreased in diabetic nephropathy subjects. This may be due to higher proportion of indirect bilirubin in serum.⁷ We also found a significant negative correlation between albuminuria and total, direct and indirect bilirubin in type 2 diabetics as well as in diabetic nephropathy subjects.

Fukui et al,¹⁰ in his study conducted among 633 type 2 diabetes patients in Japan, also found lower bilirubin levels in patients with diabetic nephropathy than patients without diabetic nephropathy, and also negative association of bilirubin with albuminuria, similar to our findings. Another post hoc analysis also showed that bilirubin is inversely associated with diabetic nephropathy progression.¹ Toya et al,¹¹ in his study found that elevated bilirubin levels were associated with decreased risk of progression from microalbuminuria to macroalbuminuria. Okada et al,¹² reported that decreased bilirubin levels might be a risk factor for the development of albuminuria in type 2 diabetes patients.

These previous studies have studied only the relationship between total bilirubin and diabetic nephropathy, but hardly two or three studies have investigated the relationship between indirect and direct bilirubin and diabetic nephropathy. Wang et al,² in his study showed that elevated total and indirect bilirubin levels significantly decreased the risk of diabetic nephropathy development in type 2 diabetes patients. Other studies conducted in type 1 diabetes patients, also showed negative correlation of total and indirect bilirubin levels with albuminuria.^{4,13}

Several mechanisms have been proposed to explain the protective role of bilirubin against the development

of albuminuria and diabetic nephropathy.⁷ Oxidative stress is one of the major mechanisms for the development and progression of diabetic nephropathy.¹ Bilirubin is found to be an endogenous anti-oxidant,^{1,14-15} and a study conducted in rodents, suggested that bilirubin protects against the development of diabetic nephropathy by inhibiting NADPH oxidase.¹ Kumar et al,¹⁶ showed negative correlation of bilirubin with oxidative stress marker, malondialdehyde and positive correlation of bilirubin with anti-oxidant enzyme activities, supporting the anti-oxidant role of bilirubin.

Bilirubin also has anti-inflammatory property by disrupting the trafficking of leucocytes to the renal interstitium, by inhibiting vascular cell adhesion protein mediated signalling. Bilirubin does so, by inhibiting the gene up regulation of endothelial adhesion molecule like E-selectin, which plays an important role in infiltrating leucocytes into renal interstitium.⁷

In addition to anti-oxidant and anti-inflammatory properties, bilirubin also has anti complement property and cytoprotective effect by inhibiting protein kinase C.^{10,17} Hence, bilirubin might prevent the development of type 2 diabetes and diabetic nephropathy by inhibiting oxidative stress and inflammation.

Conclusion

Our study suggests that low bilirubin levels might be a risk factor for the development of type 2 diabetes and diabetic nephropathy. Bilirubin being an inexpensive, easily measurable and routinely performed parameter in the laboratory can be used as a marker to predict the risk of developing type 2 diabetes among general population and also to predict the risk of developing diabetic nephropathy among type 2 diabetes patients. Further studies are required to determine whether bilirubin levels can be a therapeutic target for preventing the development of type 2 diabetes and diabetic nephropathy.

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