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Bilirubin not just a routine parameter plays an important role in chronic kidney disease

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ABSTRACT

Background: Chronic kidney disease (CKD) is a major health burden in India as well as throughout the world. Imbalance between antioxidant defense and oxidative stress plays a major role in this multifactorial disorder. Recent studies have shown that serum total bilirubin plays an important role in the progression of CKD. **Aims and Objectives:** The aim of the study is to estimate serum total bilirubin, estimated glomerular filtration rate (eGFR), urea, creatinine and to find out whether there is correlation between serum total bilirubin, eGFR, and different stages of CKD. **Materials and Methods:** The study is conducted in the department of biochemistry after getting ethical clearance. Two hundred and sixty-nine samples of controls and cases of CKD collected. Serum total bilirubin, urea, and creatinine were calculated in autoanalyzer using standard protocol. eGFR is calculated using a standard formula. **Results:** There is a significant positive correlation (spearman's correlation $r = 0.196$, $P = 0.001$) between serum total bilirubin and eGFR value. There is also a significant negative correlation ($r = -0.239$, $P < 0.0001$) between serum total bilirubin and stages of CKD. Median value of serum urea is also high in cases compared to control. **Conclusion:** The serum bilirubin may be an important biomarker in the pathogenesis of CKD.

Key words: Bilirubin; Antioxidant; Oxidative stress; Estimated glomerular filtration rate; Chronic kidney disease

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INTRODUCTION

Chronic kidney disease (CKD) is a major burden to our society throughout the world.[1] The most common causes of CKD are hypertension, diabetes mellitus, tubulo interstitial disorders, immune mediated, and hereditary kidney disease. CKD is a serious condition which ultimately leads to end-stage renal disease requiring renal replacement therapy either in the form of dialysis or renal transplantation. CKD is defined as abnormalities of kidney structure or function, present for more than 3 months, with implications for health which includes markers of kidney damage (albuminuria, albumin creatinine ratio ≥30 mg of albumin/g of creatinine, urine sediment abnormalities, electrolyte and other abnormalities due to tubular disorders, abnormalities detected by histology, structural abnormalities detected by imaging) or decreased glomerular filtration rate (GFR) (GFR <60 mL/min/1.73 m^2).[2,3] Over the course of CKD, patients progress through several stages (G1, G2, G3a, G3b, G4, and G5). Several studies indicate that free radicals and inflammation play an important role in progression CKD.[4,5] Living organism requires oxygen for their survival. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed from molecular oxygen due to aerobic metabolism. ROS includes radicals such as hydroxyl radicals, superoxide, peroxyl, alkoxyl, and hydroperoxyl radicals as well as non-radical species such as hydrogen peroxide, peroxynitrite, singlet oxygen, hypochlorous acid.[6] RNS include radicals such as nitric oxide (NO), nitrogen dioxide as well as non-radicals such as nitrous acid, peroxynitrous acid.[7] These compounds can attack deoxyribonucleic acid (DNA), protein and even lipids producing deleterious effects. To counterbalance these toxic compounds,

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humans are equipped with antioxidant defense system. These include agents that catalytically remove free radicals (catalase [CAT], glutathione peroxidase, superoxide dismutase [SOD], glutathione reductase, and thiol specific antioxidants), proteins that minimize the availability of pro oxidants (transferrin, haptoglobulin, haemopexin, and metallothionein), and low molecular weight agents that scavenge ROS and RNS such as reduced glutathione, alpha-tocopherol, uric acid, and bilirubin.[8] When the balance between oxidants and antioxidants shifts toward oxidants, oxidative stress is developed. Oxidative stress has been implicated in cardiovascular disease, diabetes mellitus, neurological disease, cancer, and even in CKD. Oxidative stress is widely considered a biochemical hallmark in CKD influencing the gradual decline in renal function and onset of major systemic comorbidities.

CKD is associated with progressive loss of renal function. Gradual decline of renal function leads to the accumulation of toxic substances can be excreted by the kidney in normal conditions. These uraemic toxins play a crucial role in oxidative stress and inflammation in CKD.[9] Despite regular dialysis, incomplete removal of these waste products leads to the accumulation of uraemic toxins, which further aggravate the condition. Among these toxins, protein-bound toxins such as indoxyl sulfate, p-cresyl sulfate play crucial roles. Most of these protein-bound toxins are gut derived and are the byproducts of aromatic amino acid breakdown by intestinal bacteria. Other sources of ROS are mainly the mitochondrial respiratory chain and the high activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. In addition increased NO and markers of oxidative stress-such as malondialdehyde, peroxynitrite ($ONOO^-$) and advanced glycation end products (AGEs) interact with AGE receptors and activate nuclear factor Kappa B pathway, with a consequent increase in cytokines and adhesion molecules like intercellular adhesion molecule 1 which recruits monocytes in renal tissue, monocyte chemoattractant protein 1 which is a chemotactic cytokine helping in macrophage infiltration and activation of tubulointerstitial inflammation.[10] The oxidative stress is also reflected in a reduction in the activity of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) which is important in the expression of antioxidant and cytoprotective enzymes like NAD (P) H dehydrogenase [quinone] 1, CAT and SOD during stress. Thus these compounds produce nephrotoxic effects through generation of ROS, depletion of anti-oxidant enzymes, and induction of fibrosis and inflammation by modulating mediators like cytokines and transcription factors.

Bilirubin is derived mainly from two sources. Majority of bilirubin is made from the breakdown of hemoglobin in senescent red blood cells. The remainder originates from the turnover of various heme-containing proteins found in other tissues. These proteins include myoglobin, cytochromes, CAT, peroxidase, and tryptophan pyrrolase.[7] The catabolism of heme from all of the heme containing proteins is catalyzed by a complex enzyme system, heme oxygenase producing ferric ion, CO, and biliverdin. This is followed by reduction methyne bridge between pyrrole 3 and pyrrole 4 to a methylene group by biliverdin reductase to produce yellow pigment biliribin. Bilirubin binds to albumin in the circulation and is transported to the liver, where it is conjugated by uridine diphosphate-glucuronyl transferase 1A1 with glucuronic acid and excreted into bile mediated by multi-drug resistance protein-2. Once entering the bile canaliculi, conjugated bilirubin is stored and mixed with the other constituents of bile in the gall bladder. The stored bile is propelled into the duodenum to facilitate chemical digestion.

Bilirubin is generally considered as useless metabolite with little physiological function. Like Roman god Janus Bifrons, bilirubin has two faces in human. When elevated excessively causes neurotoxicity, low level of bilirubin is also dangerous. In recent years, it has been seen that higher serum bilirubin level within physiological range is associated with lower risk of development and progression of CKD. This may be due to its potent antioxidant, anti-inflammatory, and cytoprotective activity.[18] Bilirubin is the powerful scavenger of peroxyl radicals and singlet oxygen.[13] Bilirubin also directly inhibits NADPH oxidase activity and suppressed superoxide generation in vascular endothelial cells as well as in renal tubular cells.[14,15] Similarly, both endogenous and exogenous bilirubins have been shown to attenuate oxidative stress and renal dysfunction in animal studies. Oh et al., demonstrated that intraperitoneal administration of bilirubin markedly improved tubular injury and interstitial fibrosis through protection from oxidative stress and apoptosis in a rat model of cyclosporine nephropathy.[16] Chronic low-grade inflammation is common in patients with CKD. The causes of inflammation in CKD are multifactorial, including increased production of pro-inflammatory cytokines, uremic toxins and oxidative stress, and the dialysis procedure itself. Persistent inflammation can lead to the progression of kidney disease, and development of complications of CKD. While oxidative stress and inflammation are key factors of CKD progression, Nrf2 confers protection against kidney damage by inducing antioxidant responses to oxidative stress. Recent studies have shown that bilirubin is a potent endogenous compound that activates Nrf2 pathway under conditions of oxidative stress.[17,18] Transcription factor Nrf2 plays a key role in regulating the expression of antioxidant genes by binding with antioxidant response element in DNA. Anti-inflammatory property of bilirubin is due to inhibition of cell adhesion molecules expression as well as the inhibition of release of IL-2, IL-6, IL-10, and TNF

alpha. Keum et al., demonstrated that bilirubin nanoparticles can cause significant anti-inflammatory activity against various oxidative stress-related disorders.[19] Renal fibrosis is another important finding in CKD. Research has shown that bilirubin reduces induction of pro fibrotic marker like HIF-1 alpha.[12] Podocyte apoptosis is another important feature in CKD. Decreases in podocyte numbers are associated with further decline in GFR. Bilirubin also has antiapoptotic effect.[12,20]

Aims and objectives

In this present work, the objective behind is to compare serum total bilirubin with estimated GFR (eGFR) and stages of CKD and to find out if there is any correlation among them in CKD patients.

MATERIALS AND METHODS

Two hundred and sixty-nine numbers of CKD patients attending nephrology outpatient department of Calcutta National Medical College, Kolkata, and 269 control subjects are included in our study after obtaining informed consent with prior approval from the Institutional Ethics Committee, Calcutta National Medical College letter number(EC-CNMC/103 dated September 24, 2022). 5 mL of blood is collected from each patient and centrifuged at 2500 rpm for 5 min. Serum is separated and kept in refrigerator at −4°C and tests are performed in the next day (serum urea, creatinine, and total bilirubin). eGFR is calculated using CKD-epidemiology collaboration (CKD-EPI) equation 2021. CKD-EPI Creatinine equation Expressed as a single equation eGFR=142×min(Scr/κ,1) α×max(Scr/κ,1)-1.200×0.9938 age×1.012 (if female) Abbreviations/Units SCr eGFR =mL/min/1.73 m^2 (standardized serum creatinine)=mg/dL κ=0.7 (females) or 0.9 (males) α=−0.241 (females) or −0.302 (males) min=indicates the minimum of SCr/κ or 1 max=indicates the maximum of SCr/κ or 1 age=years. All the tests are performed in the department of biochemistry, CNMCH.

Inclusion criteria

CKD is defined as abnormalities of kidney structure or function, present for >3 months, with implications of health. We chose those patients who have GFR value <60 mL/min/1.73m^2.

Exclusion criteria

Following patients are excluded from the study:

1. Recent history of acute kidney injury
2. Recent acute cardiovascular event
3. Kidney transplant patients, malignancy, and active infection.

Estimation of serum urea is done by glutamate dehydrogenase method,[21] estimation of creatinine is done by Jaffe's kinetic method[22] and estimation of serum total bilirubin is done by DPD method[23] using standardized kit using autoanalyzer. eGFR is calculated using CKD -EPI formula by putting the values of age, sex, and serum creatinine values.

RESULTS

In our study, data are extracted and statistical analysis is done using Microsoft Office Excel 2007 and SPSS Statistics version 2020. The values of serum creatinine and urea are significantly higher than the healthy controls and value of serum total bilirubin is significantly lower in CKD patients compared to control group. CKD patients are subdivided in 5 stages depending on GFR values. Stage 1 with normal or high GFR >90 mL/min, Stage 2 GFR 60–89 mL/min, Stage 3a GFR 45–59 mL/min, Stage 3b GFR 30–44 mL/min, Stage 4 GFR 15–29 mL/min and Stage 5 with GFR <15 mL/min.

Next comparison of serum total bilirubin between control and different stages of CKD has been made to show the relationship between them. Figure 1 depicts that there is a gradual decline of serum total bilirubin as the stage of CKD advances. Table 1 shows demographic and biochemical values among control and cases. The frequency and the percentage of CKD cases of different stages are shown in Table 2.

Table 3 shows different variables among the stages of CKD patients. Values are expressed in median as they are non-normally distributed.

The Table 4 shows a correlation among the variables. There is a significant positive correlation (r=0.196, P=0.001) between serum total bilirubin and eGFR value.

The Table 5 shows the strength of association between serum total bilirubin and stages of CKD. Table 6 shows

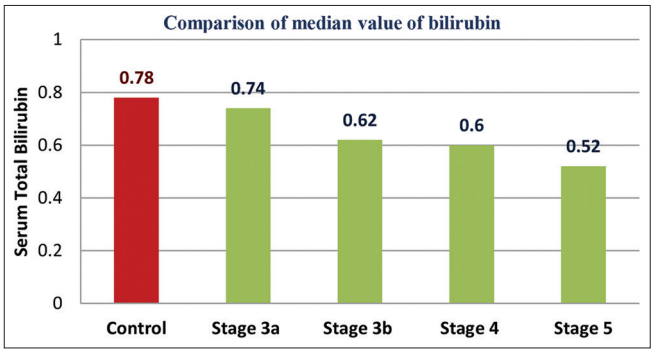

Figure 1: Gradual decline in serum total bilirubin as stage of CKD advances

Table 1: Different demographic and biochemical values (Mean, SD, Median, IQR) of different parameters among controls and cases

Dataset	Number	Sex	Age		Total Bilirubin		Total Bilirubin		Urea		Creatinine		Creatinine		eGFR		eGFR	
			Mean	Median	Mean		Median		Mean		Median		Mean		Median		Mean	
			SD	IQR	SD		IQR		SD		IQR		SD		IQR		SD	
Controls	269	Male 192 71.90% Female 75 28.10%	56.24 9.18	56 14 (63–49)	0.78 0.2		0.76 0.32 (0.94–0.62)		25.6 9.03		24 14 (32–18)		0.94 0.16		0.96 0.24 (1.06–0.82)		87.03 15.41	86 26 (100–74)
Cases	269	Male 192 71.90% Female 75 28.10%	57.32 11.76	55 15 (65–49)	0.59 0.18		0.59 0.26 (0.70–0.44)		98.28 40.8		96 62 (127–65)		3.49 1.45		1.98 0.24 (4.3–2.32)		21.46 9.68	20 14 (28–14)

SD: Standard deviation, IQR: Interquartile range, eGFR: Estimated glomerular filtration rate

Table 2: Frequency and percentage of CKD cases of different stages in study

CKD stages	Frequency	Percentage
3a	7	2.6
3b	50	18.6
4	135	50.2
5	77	28.6

CKD: Chronic kidney disease

Table 3: Different variables among stages of CKD patients

Variables	Stage 3a	Stage 3b	Stage 4	Stage 5
Urea	58	62	85	131
Creatinine	1.7	2.14	3	4.95
eGFR	48	35	20	12
Total Bilirubin	0.74	0.62	0.6	0.52

CKD: Chronic kidney disease, eGFR: Estimated glomerular filtration rate

Table 4: To check the correlation among the variables spearman's correlation is done

Variables	Total Bilirubin	
	Spearman's correlation (r)	P-value
eGFR	0.196	0.001
Age	0.045	0.466

eGFR: Estimated glomerular filtration rate

Table 5: Checks strength of association between total bilirubin and different stages of CKD

Correlations			
Spearman's rho		Bil	Stage
Bil	Correlation Coefficient	1	−0.239**
	Sig (2-tailed)	0.0	0
	n	269	269
Stage	Correlation Coefficient	−0.239**	1
	Sig. (2-tailed)	0	0.0
	n	269	269

**.Correlation is significant at the 0.01 level (2-tailed), CKD: Chronic kidney disease

Table 6: Correlation between total bilirubin and stages of CKD

Variables	Total Bilirubin	
	Spearman's correlation (r)	P-value
Stages of CKD	−0.239	<0.0001

CKD: Chronic kidney disease

a significant negative correlation (r=−0.239, P<0.0001) between serum total bilirubin and stages of CKD.

DISCUSSION

In our study, we found that the serum total bilirubin level is gradually decreasing (still within normal range) as

eGFR decreases. There is a significant positive correlation (spearman's correlation r=0.196, P=0.001) between serum total bilirubin and eGFR value in our study. Shin et al., also found a positive correlation between serum total bilirubin and eGFR (r=0.128. P=0.0001) in their study in Korean population.[25] Katoh et al., also found a positive correlation r=0.22, P<0.001) in their study.[24] Another interesting finding is that serum total bilirubin is negatively correlated with stages of CKD (spearman's correlation r=−0.239, P<0.0001) in our study. Liu et al.,[26] have also shown that lower serum bilirubin within a normal physiological range has poor outcome on stages of CKD. We have also found that the median value of serum urea is much higher in CKD patients compare to control groups. Urea itself is a toxin. Carbamylation is the process of protein modification by isocyanic acid, a breakdown product of urea.[27] Carbamylated albumin may stimulate interstitial fibrosis in kidney. Bilirubin is an important biomarker for the progression of CKD.[28] It may be a therapeutic target for the prevention of CKD in near future because of its antioxidant, anti-inflammatory, immunomodulatory, anti-apoptotic property. Further investigations are required to comment whether total bilirubin concentration is a potential therapeutic target for the prevention of CKD or not.

Limitations of the study

If the sample size increases, the result can provide more clear understanding about the role of bilirubin in progression of CKD.

CONCLUSION

In this study the serum bilirubin level decreases with the progression of difference stages of CKD. Thus, serum bilirubin may play an important role as a biomarker in the pathogenesis of CKD.

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SS- Conceptualization, design of the study, and drafting of manuscript; **SB**- Data analysis, supervision, and final approval; **SL**- Data analysis and review of manuscript; **SD-** Test performance and review of manuscript.

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