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Original Article

Serum bilirubin as a predictor of graft outcomes after renal transplant

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Abstract: Bilirubin is a signaling molecule that alters the immune response and metabolism. While bilirubin has been employed as a marker of renal and cardiovascular health, its role in renal transplant recipients is not known. In this study, we sought to determine the impact of bilirubin (total, direct and indirect) on the estimated glomerular filtration rate (eGFR) after renal transplantation. We conducted a retrospective review of pre- and postoperative bilirubin levels in 457 renal transplant recipients at a single center. Pre- and post-rejection bilirubin levels were also assessed in those patients who experienced a rejection episode. No statistically significant differences were found in bilirubin levels during the pre-transplant to post-rejection period among patients who experienced rejection with kidney allograft survival. No statistically significant associations were observed between baseline bilirubin and post-transplant eGFR in the full patient group or within the gender- or race-stratified groups. Baseline bilirubin was not correlated with time to rejection. Our results suggest that bilirubin may not offer renoprotection in renal transplant recipients.

Keywords: Kidney transplant, bilirubin, nephroprotection, GFR, renoprotection

Introduction

Bilirubin is a byproduct of heme catabolism and is an indicator of liver dysfunction [1]. Bilirubin occurs in two forms: indirect bilirubin (Ibili) and direct bilirubin (Dbili). Ibili is the dominant form and is bound to circulating albumin in an unconjugated form [2]. Hepatocytes convert Ibili into Dbili by conjugation with glucuronide [2]. Conjugated bilirubin (Dbili) is water soluble and is secreted into the bile, while unconjugated bilirubin (Ibili) is not water soluble. Total bilirubin (Tbili) values represent the sum of Dbili and Ibili. Clinically, Tbili and Dbili levels are measured, and indirect bilirubin levels are calculated as Ibili = Tbili - Dbili.

Historically, bilirubin was considered merely as a waste product of heme metabolism that is excreted by the body. However, more recent studies have suggested that bilirubin has antioxidant and anti-inflammatory properties [3]. Additionally, bilirubin has been found to exhibit a renoprotective role in multiple studies [3-5]. One study noted a positive correlation between total serum bilirubin concentrations and eGFR [6]. However, two studies [7, 8] found that increased serum bilirubin concentration was a risk factor for the progression of chronic kidney disease. Another report [9] concluded that elevated Tbili was associated with decreased eGFR. Our group has shown that higher pre- and post-bilirubin preserved graft function and enhanced allograft survival in transplant recipients in an animal model [10].

In this study, we assessed the potential correlation of serum bilirubin and renal function in a cohort of transplant recipients to resolve the conflict surrounding the effects of bilirubin on transplanted kidneys in the clinic. We hypothesized that higher baseline bilirubin levels in renal transplant recipients would be associated with higher post-transplant eGFR, decreased graft rejection, and faster recovery from rejection. A secondary objective was to determine

the impact of gender and African American race on the correlation of bilirubin to eGFR.

Methods

Inclusion and exclusion criteria

We conducted a retrospective review of the renal transplant recipient cohort (n=457) at the University of Toledo Medical Center from December 1, 2014 to April 31, 2021. Transplantation was indicated for diabetic nephropathy, glomerulonephritis, pyelonephritis, polycystic kidney disease, hemolytic uremic syndrome, Goodpasture's syndrome, and IgA nephropathy in our patient population.

Inclusion criteria for our study:

-All consecutive primary renal transplant recipients who received their first allograft at the University of Toledo Medical Center from 12/1/14-4/31/21, who had post-transplant follow up for at least one year

-All etiologies of renal failure

-Adults >18 years

-Creatinine clearance equal to or less than 20 mL/min

-No active or ongoing cancers

-No active infections

-Reasonable heart function in a patient with known cardiovascular disease as determined by a cardiologist

-Reasonable pulmonary function in a patient with known pulmonary disease as determined by a pulmonologist

-BMI equal to or less than 40

-No use of tobacco, alcohol, or illegal substances

-Documented compliance with treatment modalities

Exclusion criteria for our study:

-History of previous renal transplantation

-HIV with active AIDS

-Metastatic carcinoma

-Less than one year of follow up data

-Pediatric patients

-Other solid organ transplantations: liver, heart, bowel etc.

Patients with less than one year of post-transplant follow-up or who had undergone a previous transplant were excluded. Otherwise, all patients who underwent a renal transplant at our center were included.

Demographics

Relevant variables including patient age, sex, race, cytomegalovirus (CMV) status, and allograft rejection status were collected from the patient charts. In all cases, the diagnosis of rejection was established by renal transplant biopsy. All biopsies were performed only to evaluate unexplained graft dysfunction and suspected rejection, as our center does not perform biopsies as part of the protocol.

Outcome measures

Tbili and Dbili were measured at the UTMC Pathology clinical laboratory using the Beckman Coulter AU Analyzer [11]. Reference ranges for the measurements were as follows: Tbili (0.3-1.0 mg/dL), Dbili (0.03-0.18 mg/dL), and Ibili (0.27-0.82 mg/dL). Tbili and Dbili measurements at four weeks and two weeks prior to transplant, as well as at two and four weeks postoperatively were collected for each patient. If rejection occurred, an additional set of labs were collected at four and two weeks before and after the date of rejection diagnosis. Ibili was calculated as Tbili - Dbili. eGFR was measured using the Modification of Diet in Renal Disease (MDRD) method [12].

Statistical analysis

All data were analyzed using SPSS v27 [13]. Descriptive statistics were calculated including means and standard deviations or medians and interquartile ranges for continuous variables and Ns and percentages for categorical variables. Demographic and clinical characteristics were compared by rejection status using independent sample t-tests, Mann-Whitney U, and chi-squared tests as appropriate. Repeat-

Table 1. Demographic and clinical characteristics of transplant recipients

	Experienced Rejection N (%)	Did not experience rejection N (%)	*P*-value
Age at first transplant (mean (SD))	47.5 (15.4)	55.6 (13.1)	<0.001
Male	30 (57.7)	263 (64.9)	0.31
African American	15 (28.8)	104 (25.7)	0.62
CMV positive donor	25 (49.0)	184 (46.0)	0.68
CMV positive recipient	29 (56.9)	211 (52.9)	
Days of follow-up or time to first rejection (Median (IQR))	614 (286-1081)	103 (23-265)	<0.001
Last eGFR taken on patient (mL/min)			<0.001
≥60	11 (21.2)	220 (54.3)	
45-60	14 (26.9)	99 (24.4)	
30-44	13 (25.0)	57 (14.1)	
15-30	8 (15.4)	15 (3.7)	
<15	6 (11.5)	14 (3.5)	

ed measures ANOVA was used to examine changes in bilirubin over time. Associations between bilirubin and eGFR were examined via one-way ANOVA and the correlation between baseline bilirubin and time to rejection/last day of observation was examined with Spearman correlation analysis.

Ethics statement

The retrospective study was approved by the Institutional Review Board at the authors' institution (University of Toledo Institutional Review Board, Reference #300243-UT) and was performed in accordance with the Helsinki declaration.

Results

Patient demographics

A total of 457 patients met the inclusion criteria, of which 64.1% were male and 35.9% were female. The average age at transplantation for participants was 54.7 years. Most patients identified themselves as non-African American (74.0%). Deceased donors were the source of most kidneys (86.0%). Patients experiencing transplant rejection tended to be older than those that did not experience rejection (P<0.001), but similar with respect to gender, African American race percentage, and CMV donor and recipient status (**Table 1**).

Rejection and CMV status

Fifty-two patients had biopsy-confirmed rejection (11.4%), of which the majority (47/52 patients; 90.4%) had T-cell-mediated rejection while 9.6% (5/52 patients) had B-cell-mediated rejection. After kidney transplantation, active CMV infection has been associated with higher risk of kidney allograft failure [14]. Hence, CMV status and correlation with rejection were assessed. CMV status for the Donor (D) and the Recipient (R) were: D+R+ (29.9%), D-R+ (23.1%), D+R- (16.4%), and D-R- (30.2%). CMV status correlated with rejection as follows: CMV positive donors with rejection (49%), CMV positive recipients with rejection (56.9%), CMV positive donors without rejection (46%), and CMV positive recipients without rejection (52.9%). CMV data were missing for eight patients.

Outcomes

Baseline total bilirubin levels differed between African American and non-African American patients, and total and indirect bilirubin levels differed when stratified by gender (**Table 2**). Mean pre-transplant and post-transplant bilirubin levels are summarized in **Table 3**. Pre-and post-transplant means were not statistically different between patients that experienced rejection and those that did not. Repeated measures ANOVA did not indicate a statistically significant trend of change in total bilirubin during the pre-transplant to post-rejection period among patients that experienced rejection. No statistically significant associations were observed between baseline bilirubin and post-transplant eGFR in the full patient group, or the gender- or race-stratified groups. Baseline bilirubin levels were not correlated with time to rejection.

Table 2. Race and gender associations with baseline bilirubin

	Mean Bilirubin (mg/dl)	Significance
Race vs Direct Bilirubin		
Non-African American (n=222)	0.10±0.06	P=0.326
African American (n=66)	0.09±0.07	
Race vs Indirect Bilirubin		
Non-African American (n=223)	0.43±0.18	P=0.433
African American (n=66)	0.41±0.19	
Race vs Total Bilirubin		
Non-African American (n=338)	0.52±0.20	P=0.047
African American (n=119)	0.48±0.18	
Gender vs Direct Bilirubin		
Male (n=191)	0.11±0.06	P=0.089
Female (n=97)	0.09±0.06	
Gender vs Indirect Bilirubin		
Male (n=191)	0.45±0.20	P<0.001
Female (n=98)	0.38±0.15	
Gender vs Total Bilirubin		
Male (n=293)	0.54±0.20	P<0.001
Female (n=164)	0.46±0.15	

Discussion

Results from the kidney transplant patient cohort at our center do not indicate that bilirubin exerts protective or detrimental effects on kidney allograft function and survival. In fact, bilirubin levels did not appear to be associated with allograft survival and eGFR values. These results are novel in view of the lack of medical literature on the effect of bilirubin on kidney allograft survival. Furthermore, no randomized control trials have been performed to assess the changes in serum bilirubin levels after kidney transplantation. However, a few earlier studies have suggested that bilirubin may prevent various kidney related illnesses including diabetic nephropathy [15], chronic kidney disease [4, 5, 16, 17], fibrosis-related renal disease [18], and renal disease in cardiac patients [19]. Interestingly, the different components of bilirubin have been proposed to have individual effects in the overall protective function. One study [20] concluded that Ibili prevented KD while Tbili and Dbili did not. Another study [21] found that better kidney function was associated with higher Ibili and Tbili levels but not with Dbili in a diabetic kidney disease patient cohort. A recent retrospective study [22] found that graft and patient survival was inversely associated with serum bilirubin levels; however, bilirubin was not fractionated into the conjugated and unconjugated fractions in this study. Moreover, the results may be confounded by the higher proportion of living donors and relatively younger age of the recipients.

A large body of evidence on the protective effects of bilirubin is derived from research conducted in East Asian countries. Higher bilirubin levels in Taiwanese patients were correlated with decreased risk of kidney failure, while also being protective against end-stage kidney disease in Korean patients [9, 23]. The inverse was also true as low Tbili was correlated with an increased risk of kidney dysfunction [24].

Despite the evidence in support of the protective effect of bilirubin in various kidney diseases such as CKD and diabetic nephropathy, our results do not indicate a protective or detrimental effect on kidney allograft function and survival. We found that bilirubin levels did not correlate with allograft survival and eGFR. There may be many reasons for the discrepancy. One reason may be that bilirubin, despite being an overall protective molecule for kidney function, is not protective in a renal transplantation setting. In fact, bilirubin levels may rise due to an underlying subacute rejection or vasculitis which might increase inflammatory markers and, thereby, modify liver function [25, 26]. Immunosuppressive drugs used in transplant procedures are metabolized by the liver and, therefore, may alter the function of bilirubin [27]. Other medications, such as steroids, may also influence bilirubin function [28]. Finally, our findings may stem from differences in the populations studied. Most prior reports involved East Asian cohorts, while our study utilized US patients. As mentioned earlier, two studies [7, 8] in US patients found that bilirubin was a risk factor for depressed eGFR as well as worse CKD outcomes. Consequently, race, ethnicity and bilirubin may influence each other in a more complex and intricate manner in a kidney transplantation scenario.

Table 3. Mean bilirubin measurements (mg/dl) at various time points among patients that did and did not experience rejections

Time point	Experienced Rejection Mean (SD)			Did not experience rejection Mean (SD)		
	Total Bilirubin	Direct Bilirubin	Indirect Bilirubin	Total Bilirubin	Direct Bilirubin	Indirect Bilirubin
Pre-transplant	0.54 (0.25)	0.11 (0.08)	0.42 (0.17)	0.50 (0.18)	0.10 (0.06)	0.49 (0.24)
Post-transplant	0.44 (0.15)	0.10 (0.05)	0.33 (0.12)	0.45 (0.18)	0.11 (0.08)	0.37 (0.17)
Pre-rejection	0.79 (1.28)	0.14 (0.11)	0.67 (1.35)	-	-	-
Post-rejection	0.49 (0.21)	0.13 (0.09)	0.37 (0.18)	-	-	-

Our study also found higher levels of Tbili (P=0.047) among non-African American patients relative to African American patients, which corroborates prior reports [29, 30]. Clinically, non-African Americans are reported to experience lower rejection rates compared to [3, 7] African American transplant recipients, which may suggest a protective role of Tbili. However, other factors such as access to healthcare, socioeconomic status, distrust of healthcare systems, alcohol consumption, dietary habits, and genetic differences may complicate the clinical picture [31]. Furthermore, male transplant patients in our cohort were found to have significantly higher Tbili and Ibili levels in relation to female transplant patients. Healthy males, in general, exhibit higher eGFR values compared with healthy females. Male patients in our patient cohort did exhibit higher eGFR values, but the differences were not statistically significant (P= 0.083) [32]. The male gender has also previously been described as an independent risk factor for decreased graft survival [33]. Future randomized studies are needed to confirm the gender-based differences.

Limitations of this study include the inherent weaknesses associated with a retrospective study of a modest cohort originating from a single center. Strengths include the high rate of patient follow-up (all patients had pre- and post-operative labs available for analysis).

In conclusion, contrary to published results showing that bilirubin offers renoprotection in native kidneys and animals, our findings suggest that bilirubin may not offer renoprotection in transplant recipients. Additional studies are needed to understand the complex relationship between bilirubin and graft survival after renal transplantation.

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Disclosure of conflict of interest

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