

# Relationship Between Serum Indirect Bilirubin Levels and Cardiovascular Events and All-Cause Mortality in Maintenance Hemodialysis Patients

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**Purpose:** Unconjugated bilirubin is one of the most endogenous antioxidant substances. Mildly elevated total bilirubin concentrations may protect against cardiovascular disease and total death. However, most studies only focused on the association between serum total bilirubin and the risk of cardiovascular disease and total death. This study aimed to investigate the relationship between serum indirect bilirubin (IBIL) and the cardiovascular events in maintenance hemodialysis patients.

**Patients and Methods:** This retrospective cohort study included 284 maintenance hemodialysis patients. Patients were divided into two groups according to the median IBIL level: high IBIL group (IBIL  $\geq 3.0$   $\mu\text{mol/L}$ ) and low IBIL group (IBIL  $< 3.0$   $\mu\text{mol/L}$ ). All demographic and laboratory data were recorded at baseline. The endpoint was cardiovascular events and all-cause mortality.

**Results:** During the median follow-up time of 62 months, 96 patients developed cardiovascular disease. There were 134 deaths. In Kaplan–Meier analysis curves, the risk of cardiovascular events in the low IBIL group was significantly higher than high IBIL group ( $P < 0.001$ ). In multivariate Cox regression analysis, the risk of cardiovascular events in high IBIL group was 0.484 times (95% CI 0.278–0.844,  $P = 0.010$ ) the risk in low IBIL group. However, there was no significant association between serum IBIL level and all-cause mortality ( $P = 0.269$ ).

**Conclusion:** Our findings suggest that lower circulating IBIL levels were associated with the increased risk of cardiovascular events in maintenance hemodialysis patients.

**Keywords:** cardiovascular disease, bilirubin, hemodialysis, retrospective study

## Introduction

Between 40% and 50% of deaths among patients with end-stage renal disease (ESRD) are due to cardiovascular causes, and the risk of cardiovascular mortality in patients with ESRD is 8.8-fold higher than in the general population.<sup>1,2</sup> Therefore, it is an important clinical priority to find novel and innovative biomarker and potential therapeutic target to reduce cardiovascular event and improve clinical outcomes in ESRD.

Unconjugated bilirubin possesses potent antioxidant,<sup>3,4</sup> anti-inflammatory,<sup>5</sup> and possibly lipid-lowering properties.<sup>6</sup> Recent clinical studies show mildly elevated total bilirubin is associated with protection from kidney damage and dysfunction in chronic kidney disease patients,<sup>7,8</sup> and low serum total bilirubin levels are also associated with the loss of residual kidney function in peritoneal dialysis patients.<sup>9</sup> Furthermore, unconjugated bilirubin functions intracellularly, as a potent inhibitor of NADPH oxidase complexes and albumin-bound bilirubin contributes significantly to the oxidant scavenging activity of plasma and may also potentially reduce endothelial dysfunction, which is strictly related to cardiovascular disease and total death.<sup>3,4,10</sup> Similar inverse associations have now been shown between serum total bilirubin concentrations and coronary artery disease,<sup>11</sup> coronary heart disease,<sup>12</sup> peripheral vascular disease,<sup>13</sup> and stroke.<sup>14</sup> Moreover, mildly elevated total bilirubin (TBIL) concentrations may protect against cardiovascular and total death.<sup>15,16</sup> However, most previous studies only focused on TBIL without distinguishing between direct bilirubin (DBIL)

and indirect bilirubin (IBIL). Few studies investigated the relationship between serum IBIL and all-cause mortality in dialysis patients.<sup>17,18</sup> However, the relationship between serum IBIL and the cardiovascular events in hemodialysis patients remains unclear.

Therefore, in the present study, we conducted a retrospective cohort study to evaluate the relationship between serum IBIL and cardiovascular events and all-cause death in maintenance hemodialysis (MHD) patients.

## Methods

### Study Design and Population

We conducted a retrospective single-center cohort study. The study population consists of 284 patients aged over 18 years with ESRD receiving maintenance hemodialysis for at least 3 months from Shanghai Fifth People's Hospital. Study subjects were recruited between January 01, 2003, and May 30, 2020. Up to January 2022, the median follow-up time was 62 months. All of the patients were subjected to a standard bicarbonate dialysis session. Hemodialysis was performed 3 times weekly using single-use dialyzers with a membrane surface area of 1.5 to 1.7 m<sup>2</sup>. Exclusion criteria were (1) weekly dialysis for less than 12 hours; (2) baseline conditions of malignancy, infectious disease, or sepsis; (3) patients with chronic liver disease or cirrhosis; (4) patients with missing bilirubin levels (Figure 1).

The endpoint was cardiovascular event and all-cause death. The cardiovascular event was defined as ischemic heart disease, cerebrovascular disease, peripheral vascular disease, heart failure, cardiac arrhythmia, and cardiac arrest. All-cause death was defined as any death. The study was performed according to the principles of the Declaration of Helsinki of 1975, revised in 2013, and the protocol of this retrospective single-center cohort study was approved by the Ethics Committee of Shanghai Fifth People's Hospital of Fudan University (approval number 2018–145).

### Data Collection

Baseline demographic data, clinical data and biochemical parameters were collected at the initiation of hemodialysis therapy. The baseline blood samples of each patient were collected at the initiation of hemodialysis therapy. These data were complemented by clinical assessment of blood pressure (BP), and fasting blood glucose. Diabetes was diagnosed on the basis of the World Health Organization criteria. Hypertension was defined as BP >140/90 mmHg and/or the use of antihypertensive medication.

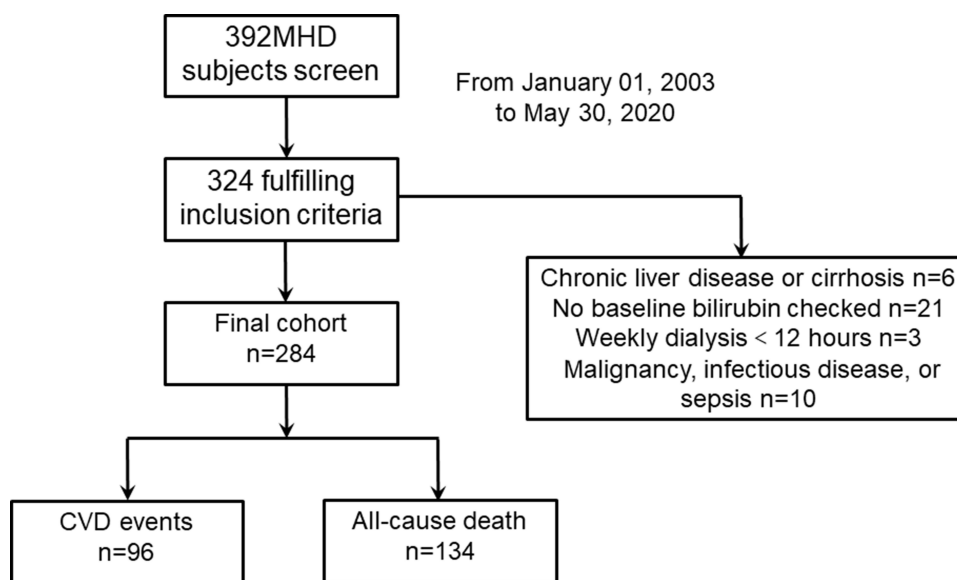


Figure 1 Study flow.

## Statistical Analysis

The Kolmogorov–Smirnov test was used to check the normality of the data distribution. Data are expressed as means  $\pm$  SD for continuous parametric data, medians and interquartile ranges for continuous nonparametric data, and frequencies for categorical data. Potential differences among the two groups were assessed with *t*-test for normally distributed data, Mann–Whitney *U* for nonnormally distributed data. Cumulative survival curves for the cardiovascular disease and all-cause mortality were compared with the Log rank test, and the survival rate was calculated by the Kaplan–Meier method. The Cox proportional hazards model was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for the risk of cardiovascular disease, initially without adjustment and subsequently adjusting for clinical parameters. For all tests, *P* values of  $<0.05$  were considered statistically significant. All statistical analyses were performed using GraphPad Prism 9 (GraphPad Software Inc., San Diego, USA) and IBM SPSS software (SPSS Inc., Chicago, USA).

## Results

### Baseline Characteristics

The study population consists of 284 MHD patients. In order to ensure that the two groups have roughly the same number of people and get enough evidence, the patients were divided into the following two groups according to the median IBIL value ( $3.0 \mu\text{mol/L}$ ): I1 (low IBIL group;  $<3.0 \mu\text{mol/L}$ ) and I2 (high IBIL group;  $\geq 3.0 \mu\text{mol/L}$ ). There were 139 patients in the I1 group and 145 patients in the I2 group. The baseline characteristics and clinical outcomes of the study population are displayed in Table 1. In the I2 group, serum TBIL, DBIL, IBIL and serum albumin and calcium values were higher; MHD duration was longer, compared with those in the I1 group.

**Table 1** Baseline Characteristics of Chronic Hemodialysis Patients Classified by the Serum Indirect Bilirubin Median Value

Characteristics of Patients	All Participants	Serum Indirect Bilirubin Concentration		P-value
		I1: $<3 \mu\text{mol/L}$	I2: $\geq 3 \mu\text{mol/L}$	
N	284	139	145	-
Men, n(%)	162(57.0%)	84(60.4%)	77(53.8%)	0.259
Age (yr)	59.82 $\pm$ 13.93	58.79 $\pm$ 13.83	60.80 $\pm$ 14.01	0.222
HD vintage(months)	62(40–101)	58(38–94)	71(43–111)	0.019
Hypertension	163(57.4%)	78(56.1%)	85(58.6%)	0.669
Diabetes mellitus	82(28.9%)	44(31.7%)	38(26.2%)	0.595
Previous coronary artery disease	23(8.1%)	11(7.9%)	12(8.3%)	0.911
Previous cerebrovascular disease	31(10.9%)	12(8.6%)	19(13.1%)	0.228
Previous congestive heart failure	43(15.1%)	25(18.0%)	18(12.4%)	0.191
Systolic BP (mmHg)	140.1 $\pm$ 18.6	140.8 $\pm$ 18.07	139.5 $\pm$ 19.2	0.545
Diastolic BP (mmHg)	83.4 $\pm$ 11.9	84.1 $\pm$ 11.7	82.7 $\pm$ 12.1	0.317
SpKt/V	1.26 $\pm$ 0.22	1.27 $\pm$ 0.22	1.24 $\pm$ 0.21	0.338
Laboratory values				
Hemoglobin (g/L)	85(70–100)	83(65–99)	86(71.5–101)	0.132
Albumin (g/dL)	36.2(32.2–40.3)	34.9(31.4–38.6)	37.7(33.6–41.3)	0.001

(Continued)

**Table 1** (Continued).

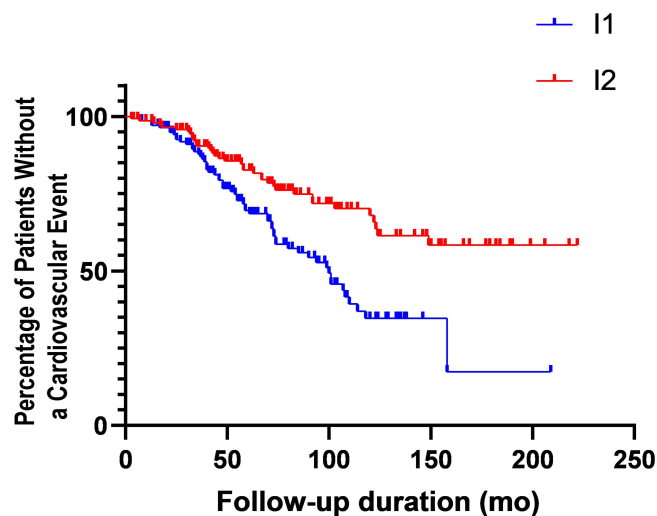
Characteristics of Patients	All Participants	Serum Indirect Bilirubin Concentration		P-value
		I1: <3 $\mu\text{mol/L}$	I2: $\geq 3 \mu\text{mol/L}$	
Creatinine ( $\mu\text{mol/L}$ )	769(626–1009)	755 (618–968)	774 (628–1019)	0.647
Uric acid( $\mu\text{mol/L}$ )	475(376–577)	476(379–599)	474(366–568)	0.324
Total cholesterol (mg/dL)	3.92 (3.31–4.78)	3.93(3.22–4.66)	3.90(3.37–4.82)	0.330
LDL-C (mg/dL)	2.30(1.72–2.93)	2.32(1.68–2.99)	2.26(1.81–2.89)	0.660
HsCRP (mg/dL)	3.4 (1–17)	4.9(1–24)	3(1–14)	0.326
Calcium(mmol/L)	2.04(1.85–2.20)	2.02(1.82–2.15)	2.10(1.86–2.25)	0.035
Phosphorus(mmol/L)	1.83(1.48–2.29)	1.90(1.52–2.30)	1.74(1.43–2.28)	0.127
Magnesium (mmol/L)	0.87(1.00–1.15)	1.00(0.87–1.13)	1.00(0.87–1.16)	0.678
TBIL( $\mu\text{mol/L}$ )	5.4 (1.9–7.0)	3.9(3.1–4.8)	6.9(5.6–8.3)	<0.001
DBIL( $\mu\text{mol/L}$ )	2.2 (1.6–3.0)	2.0(1.5–2.7)	2.3(1.8–3.1)	0.029
IBIL( $\mu\text{mol/L}$ )	3.0 (1.9–4.2)	1.9(1.3–2.4)	4.0(3.4–5.7)	<0.001
ALT (U/L)	11(7–18)	11(7–18)	11(7–18.8)	0.503
AST (U/L)	15(11–20)	15(11–21)	15(11–19)	0.714
Parathyroid hormone(pg/mL)	92.4(30.4–228)	82.9(29.7–222)	93.3(31–228)	0.759
Medication, n(%)				
CCB	88(31%)	41(30%)	47(32%)	0.595
ARB or ACEi	159(56%)	74(53%)	85(59%)	0.361
$\beta$ -receptor blocker	123(43%)	59(42%)	64(44%)	0.774
Aspirin	107(37%)	57(41%)	50(35%)	0.257
Statins	94(33%)	49(35%)	45(31%)	0.450
Clinical outcomes				
CVD event	96(33.8%)	59(42.4%)	37(25.5%)	0.003
All-cause death	134(47.2%)	64 (46.0%)	70(48.3%)	0.706

**Note:** Values expressed as mean  $\pm$  standard deviation, median, percentage or median (interquartile range).

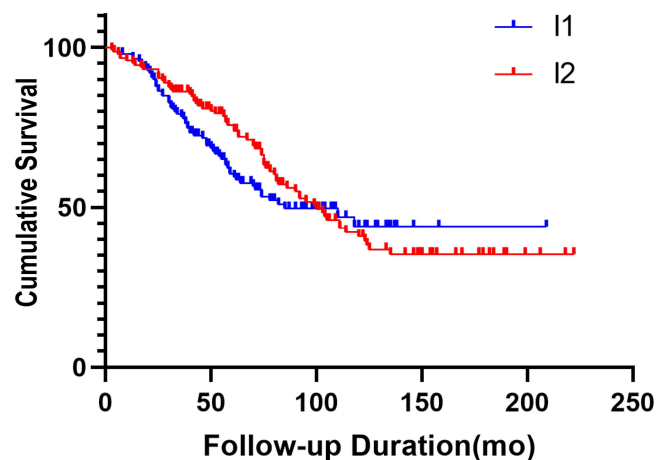
**Abbreviations:** SpKt/V, single-pool Kt/V; LDL-C, low-density lipoprotein-cholesterol; HsCRP, hypersensitive C reactive protein; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine amino-transferase; AST, aspartate amino-transferase; CCB, calcium-channel blocker; ARB, angiotensin II type I receptor blocker; ACEi, angiotensin-converting enzyme inhibitor; CVD, cardiovascular diseases.

## Survival Analysis

During the 62 months observation period, the all-cause mortality rate was 47.2% and 96 cardiovascular events occurred (Table 1). In the I1 group, 59 of the 139 patients developed cardiovascular events, while in the I2 group, 37 of the 145 patients developed cardiovascular disease. In Kaplan–Meier analysis curves for the cardiovascular events among 284 MHD patients, the risk of cardiovascular events in the I1 group was significantly higher than those in the I2 group (Log Rank  $\chi^2 = 12.97$ ,  $P < 0.001$ , Figure 2). However, there was no significant difference in the risk of all-cause mortality between the two groups (Log Rank  $\chi^2 = 2.997$ ,  $P = 0.428$ , Figure 3).



**Figure 2** Kaplan–Meier analysis curves for cardiovascular events in two groups (I1 group with serum IBIL<3.0  $\mu\text{mol/L}$ , I2 group with serum IBIL $\geq$ 3.0  $\mu\text{mol/L}$ ). The Log rank test showed a significant difference between the two groups ( $P < 0.001$ ). Symbols are: I1–; I2–.



**Figure 3** Kaplan–Meier analysis curves for all-cause mortality in two groups (I1 group with serum IBIL<3.0  $\mu\text{mol/L}$ , I2 group with serum IBIL $\geq$ 3.0  $\mu\text{mol/L}$ ). The Log rank test showed no significant difference between the two groups ( $P = 0.428$ ). Symbols are: I1–; I2–.

## Effect of IBIL Level on Cardiovascular Events

Univariate Cox regression survival analysis is summarized in Table 2. Briefly, age, serum IBIL, alanine aminotransferase and aspartate aminotransferase were the risk factors for cardiovascular events ( $P < 0.05$ ). Table 2 shows unadjusted and multivariable-adjusted HRs with 95% CIs according to baseline serum IBIL concentration as a continuous variable. In the multivariate Cox regression analysis, after adjustment for age, serum uric acid, hemoglobin, serum albumin, single-pool Kt/V (SpKt/V), hypersensitive C reactive protein (HsCRP) and the other traditional and dialysis-related risk factors, serum IBIL was also the independent risk factors for cardiovascular events (HRs = 0.773, 95% CI 0.660–0.906,  $P = 0.001$ ). In the same multivariable-adjusted model, age (HRs = 1.038, 95% CI 1.016–1.061,  $P = 0.001$ ), previous coronary artery disease (HRs = 3.839, 95% CI 1.832–8.044,  $P < 0.001$ ) and previous congestive heart failure (HRs = 1.941, 95% CI 1.021–3.692,  $P = 0.043$ ) were the independent risk factors for cardiovascular events. Consistently, there was no significant association between serum uric acid, SpKt/V, serum albumin, hemoglobin, HsCRP and cardiovascular events (Table 2). However, there was no significant association between serum IBIL and all-cause mortality (HRs = 0.818, 95% CI 0.574–1.167,  $P = 0.269$ ).

**Table 2** Risk Factors for Cardiovascular Events by the Cox Proportional Hazards Analysis in Maintenance Hemodialysis Patients

Covariate	Univariate			Multiple		
	HRs	95% CI	P-value	HRs	95% CI	P-value
Age	1.041	1.021–1.061	<0.001	1.038	1.016–1.061	0.001
Male	1.202	0.751–1.924	0.442	1.531	0.872–2.687	0.138
Hypertension	0.849	0.457–1.578	0.605	0.975	0.447–2.125	0.950
Diabetes	1.468	0.358–6.028	0.594	2.705	0.528–13.851	0.232
Previous coronary artery disease	4.341	2.443–7.714	<0.001	3.839	1.832–8.044	<0.001
Previous cerebrovascular disease	1.715	0.850–3.460	0.132	1.018	0.424–2.444	0.969
Previous congestive heart failure	1.405	1.446–4.001	0.001	1.941	1.021–3.692	0.043
Systolic Blood pressure	1.003	0.991–1.016	0.608			
Diastolic Blood pressure	1.006	0.987–1.025	0.549			
SpKt/V	0.490	0.156–1.533	0.220	0.495	0.138–1.772	0.280
Albumin	0.974	0.939–1.010	0.159	1.022	0.975–1.070	0.363
Hemoglobin	1.000	0.988–1.013	0.945	0.998	0.983–1.014	0.834
Uric acid	1.001	1.000–1.003	0.127	1.001	0.999–1.003	0.317
HsCRP	0.493	0.164–1.481	0.207	0.439	0.095–2.029	0.292
Alanine amino-transferase	1.002	1.001–1.004	0.004	0.989	0.964–1.014	0.381
Aspartate amino-transferase	1.002	1.001–1.003	0.007	1.012	0.990–1.034	0.296
Total bilirubin	0.942	0.859–1.035	0.213			
Direct bilirubin	1.117	0.977–1.278	0.106			
Indirect bilirubin	0.852	0.751–0.966	0.012	0.773	0.660–0.906	0.001
Total cholesterol	1.010	0.826–1.233	0.926	1.059	0.860–1.304	0.591
LDL-C	1.038	0.824–1.308	0.752			
Calcium	0.733	0.356–1.508	0.399			
Phosphorus	1.142	0.799–1.633	0.465	0.857	0.541–1.359	0.513
Parathyroid hormone	1.000	0.998–1.001	0.628			

**Abbreviations:** HRs, hazard ratios; 95% CI, 95% confidence interval; SpKt/V, single-pool Kt/V; HsCRP, hypersensitive C reactive protein; LDL-C, low-density lipoprotein-cholesterol.

We converted serum IBIL into binary and tertile variables when performing the Cox regression analysis (Table 3). In the multivariate Cox regression analysis, compared with the I1 group, the fully adjusted HRs in the I2 group for cardiovascular events was 0.484 (95% CI 0.278–0.844,  $P = 0.010$ ), after adjustment for age, serum uric acid, hemoglobin, serum albumin, SpKt/V, HsCRP and the other traditional and dialysis-related risk factors (Table 3). After adjustment for traditional and dialysis-related risk factors, individuals with IBIL in the upper tertile had an adjusted hazard ratio of 0.578 (95% CI 0.410–0.815,  $P = 0.002$ ) for cardiovascular disease compared with those in the lower tertile.

**Table 3** The Relationship Between Cardiovascular Events and the Baseline Serum Indirect Bilirubin by Multiple Cox Analysis

	Binary Variable			Tertile Variable		
	HRs	95% CI	P-value	HRs	95% CI	P-value
Unadjusted	0.617	0.383–0.993	0.047	0.710	0.535–0.943	0.018
Model 1 <sup>a</sup>	0.543	0.334–0.882	0.014	0.643	0.480–0.863	0.003
Model 2 <sup>b</sup>	0.489	0.296–0.806	0.005	0.617	0.458–0.832	0.002
Model 3 <sup>c</sup>	0.495	0.291–0.840	0.009	0.594	0.427–0.827	0.002
Model 4 <sup>d</sup>	0.484	0.278–0.844	0.010	0.578	0.410–0.815	0.002

**Notes:** <sup>a</sup>Adjusted for age, sex; <sup>b</sup>Adjusted for model 1 covariates and hemoglobin, HsCRP, serum albumin, SpKt/V; <sup>c</sup>Adjusted for model 2 covariates and serum uric acid, serum total cholesterol, serum phosphorus, alanine amino-transferase, aspartate amino-transferase. <sup>d</sup>Adjusted for model 3 covariates and hypertension, diabetes, coronary artery disease, congestive heart failure, congestive heart failure.

**Abbreviations:** CI, confidence interval; HRs, hazard ratios.

## Discussion

Our study demonstrated that the occurrence of cardiovascular events was negatively correlated with increasing serum IBIL binary and tertile and actual IBIL values among MHD patients followed for 62 months. This relationship also remained significant even after adjusting for potential confounding factors, including age, serum uric acid, hemoglobin, serum albumin, spKt/V, HsCRP, and serum phosphorus. This is the first report on the association between cardiovascular events and serum IBIL levels in maintenance hemodialysis patients.

Data on the association between serum bilirubin and the risk of cardiovascular events in dialysis patients are limited and inconclusive. A cohort study followed for 50 months comprised 1080 chronic hemodialysis patients demonstrated that individuals with serum total bilirubin in the upper tertile had an adjusted hazard ratio of 0.40 for cardiovascular event, compared with those in the lower tertile.<sup>19</sup> However, this research did not have unconjugated bilirubin data. In 1,419 patients with angina pectoris undergoing percutaneous coronary intervention, those with higher total bilirubin levels experienced significantly fewer MACEs (cardiac death, myocardial infarction, target-vessel revascularization, or unstable angina pectoris/heart failure, over 2.4-yr follow-up) than patients with lower total bilirubin concentrations.<sup>20</sup> Moreover, investigation of 3316 Ludwigshafen Risk and Cardiovascular Health Study participants revealed that increased total bilirubin predicted lower mortality over a period of 10.4 yr.<sup>21</sup> Furthermore, a study included 2936 subjects followed for 5.4 years showed that higher serum total bilirubin was associated with a decreased risk of developing cardiovascular death in asymptomatic diabetic patients.<sup>16</sup> However, few studies drew opposite conclusions. A retrospective cohort study included 740 peritoneal dialysis subjects followed for 28 months demonstrated all-cause mortality was higher in the patients in the higher TBIL group than in the lower TBIL group and in patients in the higher IBIL group than in the lower IBIL group.<sup>17</sup> And a nationwide retrospective cohort study investigating 47,650 hemodialysis patients followed for  $27.6 \pm 12$  months indicated that high serum total bilirubin level is associated with high mortality in patients undergoing long-term hemodialysis.<sup>22</sup> A large retrospective study investigating 1111 patients indicated that patients with ST-segment elevation myocardial infarction and higher total bilirubin undergoing percutaneous coronary intervention and stent placement had increased MACE and rate of cardiac death during their hospital admission.<sup>23</sup> The discrepancies in ethnicity and inclusion criteria may be confounding factors that caused these different results. However, most research did not have unconjugated bilirubin data, which had strong anti-oxidant effects.

Recently, two studies investigated the relationship between serum IBIL and all-cause mortality in dialysis patients. A multicenter retrospective cohort study followed for 24 months comprised 885 maintenance hemodialysis patients demonstrated that patients with  $IBIL \leq 3.3 \mu\text{mol/L}$  had hazard ratio of 1.661 for all-cause death, compared with those with baseline IBIL  $3.4\text{--}4.8 \mu\text{mol/L}$  ( $P = 0.013$ ). The Kaplan–Meier curves showed higher all-cause mortality in patients with  $IBIL \leq 3.3 \mu\text{mol/L}$  ( $P = 0.015$ ).<sup>18</sup> Zhan et al<sup>17</sup> found that there was a 56% higher risk of 5-year mortality in the higher IBIL group than in the lower IBIL group. However, our study showed that there was no significant association between serum IBIL level and all-cause mortality ( $P = 0.269$ ). The discrepancies in sample size and the follow-up time may cause different results.



The high risk of cardiovascular morbidity and mortality in MHD individuals is associated with a high prevalence of traditional risk factors for cardiovascular disease (hypertension, diabetes, and dyslipidemia). Apart from these risk factors, a series of nontraditional risk factors including calcium and phosphate abnormalities, oxidative stress, and inflammation may render ESRD patients more prone to develop excess risks of cardiovascular disease.<sup>24</sup> Up to now, many researchers have tried to assess several risk factors to improve life expectancy in ESRD patients. Unconjugated bilirubin affects atherosclerosis by several inhibiting mechanisms, including vascular smooth muscle cell proliferation, low-density lipoprotein oxidation, and endothelial dysfunction.<sup>25</sup> Additionally, elevated total bilirubin levels are associated with decreased oxidative stress status and augmented endothelium-dependent vasodilation in male Gilbert syndrome subjects who have mild unconjugated hyperbilirubinemia.<sup>26</sup> Moreover, in spontaneously hypertensive rats, the administration of hemin for 3 months elevated unconjugated bilirubin levels and total antioxidant capacity and reduced left ventricular hypertrophy, hypertension, ventricular phospholipase C activity, circulating aldosterone, and urinary excretion of oxidized lipid.<sup>27</sup> Furthermore, lipid soluble unconjugated bilirubin prevents the oxidation of cardiolipin and decreases the infarct size in the heart during ischemia.<sup>28</sup> At last, unconjugated bilirubin, which is not hydrophobic substances and plasma albumin-bound, could not be removed by hemodialysis and can keep its strong anti-oxidant effects in MHD patients. Thus, in a special group undergoing hemodialysis, high IBIL could keep protective role. In our study, in Cox regression analysis, serum IBIL levels were a risk factor for cardiovascular events ( $P < 0.05$ ). However, there was no significant association between serum DBIL, TBIL and cardiovascular disease ( $P > 0.05$ ).

Hypoalbuminemia is also a risk factor for cardiovascular disease.<sup>24,29</sup> The present study demonstrated that serum albumin value in patients with  $\text{IBIL} \geq 3.0 \mu\text{mol/L}$  was higher than that in patients with  $\text{IBIL} < 3.0 \mu\text{mol/L}$ , which may have potentially increased the protective effect of IBIL which is albumin-bound. However, in multivariate Cox regression analysis, low IBIL was still an independent risk factor for cardiovascular events in MHD patients even after adjusting for serum albumin and other potential confounding factors.

The present study has some limitations. First, this was a retrospective, relatively small sample size, single-center study that might have selection bias and reduce the statistical power. Second, we only had all-cause mortality, but we did not know the exact cause of death of the uremia patients. Third, the potential mechanism behind the relationship between serum IBIL and cardiovascular disease in MHD individuals was not clarified. Fourth, this was a retrospective study, which can only reveal associations but not causality.

## Conclusion

The current study demonstrated that lower serum IBIL concentrations were associated with the increased risk of cardiovascular events in uremia patients undergoing hemodialysis independent of established cardiovascular disease risk factors. If our findings are further confirmed by future studies, routine measurements of serum IBIL could help identify those patients at high risk of cardiovascular disease.

## Data Sharing Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

## Ethical Approval

Since this study was a retrospective one using clinical data, and it did not involve further invasive intervention, treatment, or costs to patients, the study received a consent exemption and it was approved by the ethics committee of Shanghai Fifth People's Hospital of Fudan University (approval number 2018-145).

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.



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## Disclosure

All authors have no conflicts of interest for this work.

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