Relationship between serum indirect bilirubin and prostate volume in patients with benign prostatic hyperplasia: a cross-sectional study

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Background: Due to its anti-oxidative effects, bilirubin may protect against a spectrum of diseases. However, the role of bilirubin in patients with benign prostatic hyperplasia (BPH) is poorly explored. This study aimed to investigate the cross-sectional associations between serum indirect bilirubin (IBIL) and prostate volume (PV) in patients with BPH.

Methods: The medical records of 722 BPH patients were retrospectively analyzed. Body mass index (BMI) was calculated as body weight (kg)/height $(m)^2$. PV was obtained as height (cm) × width (cm) × length (cm) × π/6. Other biochemical indexes were measured by the automatic biochemical analyzer. A univariable linear regression analysis was performed to detect confounders. The IBIL-PV relationship was examined using unadjusted and covariate-adjusted regression models. Furthermore, a segmented linear regression was conducted to analyze the linear trend of IBIL levels and PV. Finally, the sensitivity analysis was stratified by BMI and low-density lipoprotein cholesterol (LDL-C) cutoffs.

Results: In this study, the mean age of the patients was 68 (range, 43–93) years. By univariable line regression test, we observed that PV was positively correlated with age, BMI, and LDL-C (β=0.113, 0.096, and 0.135, respectively). IBIL was negatively associated with PV in full adjusted model in men age ≤75 years (β=–1.01; 95% CI: –1.81, –0.22; P=0.01). A statistically significant inverse trend was observed between serum IBIL intervals and PV in patients aged ≤75 years (adjusted for age, BMI, and LDL-C, P for trend =0.015). In sensitivity analysis, significantly negative IBIL-PV relationship only existed in men with normal BMI (adjusted β=–1.328; 95% CI: –2.467, –0.190; P=0.022), overweight men (adjusted β=–1.296; 95% CI: –2.519, –0.074; P=0.038), and men with normal LDL-C level (adjusted β=–1.017; 95% CI: –1.869, –0.164; P=0.019).

Conclusions: IBIL is negatively associated with PV in the non-obese population ≤75 years with normal LDL-C. These results suggest that higher serum IBIL possibly provides a degree of protection to BPH by mitigating oxidative stress (OS) related to aging and lipid peroxidation. Nevertheless, these preliminary findings from a single-center, retrospective study have limitations and need to be confirmed by future studies.

Keywords: Benign prostatic hyperplasia (BPH); serum indirect bilirubin (serum IBIL); low-density lipoprotein cholesterol (LDL-C); retrospective study

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Introduction

Benign prostatic hyperplasia (BPH) is the most common condition in aging men, and its incidence increases with age (1). It is characterized by the proliferation of cells in the transitional zone surrounding the urethra (2). BPH usually results in lower urinary tract symptoms (LUTS), including nocturia, dysuria, urinary urgency, weak urinary stream, and incomplete emptying (1,3), which can negatively impact patients' quality of life. Despite its universal impact, the etiology and progression of BPH remain obscure (4). As reported by numerous studies, the risk factors for BPH include age, metabolic syndrome (MetS), disturbed sex hormones, inflammation, and oxidative stress (OS) (5-7). Among these, OS plays an essential role in the pathogenesis of BPH (6,8). Vital *et al.* reported that the levels of 8-OH deoxyguanosine, a marker of OS, were higher in patients with BPH than in control patients (6).

Bilirubin is the end product of red-blood-cell (hemoglobin) breakdown. It has been confirmed as a potent antioxidant substance (9). The metabolic processes of bilirubin are summarized as follows. First, bilirubin binds to albumin (ALB) in the blood to transport to hepatocytes. This form of bilirubin is called unconjugated bilirubin (UBIL) or indirect bilirubin (IBIL) and is sparingly soluble in water under physiological conditions. Subsequently, IBIL is taken up by hepatocytes and transformed into a water-soluble form called glucuronic conjugated bilirubin (CBIL), also known as direct bilirubin (DBIL) (10). In blood circulation, IBIL accounts for the majority of total bilirubin (TBIL) levels.

Due to its antioxidant properties, bilirubin exerts protective effects in many OS-mediated diseases, such as cardiovascular disease (CVD), diabetes, cancer, and MetS (11-13). Persons with Gilbert syndrome have a circulating TBIL level >17.1 μM and have decreased prevalence of chronic diseases, particularly CVDs (14). However, little is known about the effects of bilirubin on BPH. Thus, we conducted an observational retrospective study to investigate this relationship expecting to aid in risk assessment and treatment of BPH. At the beginning of this study, age is another crucial aspect to consider, which is the most vital risk factor for BPH (15). The progression of BPH symptoms and prostate volume (PV) closely relates to older ages (15). And the etiologies of BPH may not be the same in different age groups. Therefore, our analysis of the bilirubin-PV relationship was stratified by age (aged ≤75 and >75 years). We present the following article in accordance with the STROBE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3997/rc).

Methods

Study design and participants

We retrospectively reviewed and analyzed the patient records. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Chinese PLA General Hospital (No. S2021-364-01), and individual consent for this retrospective analysis was waived. Data were collected from the Chinese PLA General Hospital's electronic medical record (EMR) system. The study included 1,247 patients admitted to the urology department of this hospital who were treated for BPH between January 2015 and February 2022. Diagnosis of BPH was confirmed by histology, radiological diagnosis, and digital rectal examinations. We excluded subjects with missing data on variables or with any medical history that may affect the PV-bilirubin relationship. The exclusion process is displayed in Figure S1. First, individuals with a history of cancer were excluded (n=63). Furthermore, individuals were excluded if they had pathological conditions affecting serum bilirubin levels, such as hepatobiliary disease (n=28), malaria (n=1), and serum alanine aminotransferase (ALT) >40 U/L (n=63). Subjects were also excluded if they had previously undergone prostatic surgery (n=29). In addition, to ensure statistical accuracy, patients without records of PV (n=91), serum bilirubin levels (n=13), or serum low-density lipoprotein cholesterol (LDL-C) levels (n=229) were excluded (n=333). Extreme outliers [> third quartile + (3 × interquartile range)] of PV (>222.23 mL, n=4) and IBIL (>23.6 μmol/L, n=4) were removed from the dataset. Finally, 722 subjects were included in this study.

Data collection

Data were collected retrospectively by reviewing the EMR. Patients' basic information, medical history, and other indicators were recorded, including age, body mass index (BMI), smoking history, alcohol consumption history, hypertension, diabetes, previous medical history, pre-operative imaging information, and routine serum biochemical indexes. The selection of indexes was based on their potential association with BPH. BPH is usually

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associated with MetS. Hence, we collected biomarkers related to lipid [triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)] and glucose (GLU) metabolism (blood GLU). On the other hand, other factors associated with OS were also obtained, including ALB, DBIL, IBIL, and serum uric acid (SUA). Serum biochemical indexes were measured using a Roche automated clinical analyzer (Roche Cobas 8000, Roche, Basel, Switzerland), including ALB, DBIL, IBIL, TG, TC, HDL-C, LDL-C, gamma-glutamyltransferase (GGT), GLU, and SUA. PV was calculated using the prostate ellipsoid formula: PV = height (cm) × width (cm) × length (cm) × π/6. BMI was calculated as BMI = body weight (kg)/height $(m)^2$.

Statistical analysis

Before analysis, occasional missing values were imputed with the regression analysis method (missing values <15). The eligible subjects were divided into two groups: those aged ≤75 years (younger group, n=590) and those >75 years (older group, n=132). Factors including hypertension, diabetes, smoking, alcohol consumption, BMI, PV, ALB, DBIL, IBIL, TG, TC, HDL-C, LDL-C, GGT, GLU, and SUA were compared in the two groups. The categorical variables were presented as % and compared by chi-square tests. Normality was tested using the Shapiro-Wilk normality test. The unpaired Student's *t*-test was performed when the variable was homogeneous and normally distributed, and the Mann-Whitney U test was used for skewed distributed variables. Normally distributed variables were expressed as mean ± standard deviation, while other continuous variables were expressed as median (quartile 1, quartile 3). First, we tested the PV-IBIL relationship using the scatter plot and Spearman's correlation using R packages ggplot2 (https://ggplot2.tidyverse.org/). To screen for potential confounders, we conducted univariable linear regression analyses using a P value of 0.05. Then, we estimated the PV-IBIL association by adopting unadjusted and adjusted models. Variables with the variance inflation factor (VIF) greater than four were excluded. Based on the normal references (0–12.4 μmol/L), IBIL was divided into three levels (0–6 μmol/L, 6.01–12.4 μmol/L, and 12.41 μmol/L or above). To determine whether there was an IBIL dose effect with PV, tests for linear trends were conducted using these levels as ordinal variables. In order to examine the heterogeneity of PV-IBIL association in different population, we adopted sensitivity analyses for BMI (normal: 18.5 kg/m^2 ≤ BMI <24 kg/m^2; overweight: 24 kg/m^2 ≤ BMI <28 kg/m^2; obesity: BMI ≥28 kg/m^2) (16) and LDL-C [cutoff =3.4 mmol/L (17)] subgroups in aged ≤75 years subjects. All statistical analyses were performed using the SPSS 26.0 software (IBM SPSS Statistics for Windows, IBM, Chicago, USA) and the R program 3.5.1 (R Development Core Team, Vienna). Statistical significance was set at P<0.05 (two-sided).

Results

Basic characteristics of the included patients

The average age of the eligible 722 patients was 68 years, which ranged from 43 to 93. We divided the total subjects into two groups: the younger group (age ≤75 years, n=590) and the older group (age >75 years, n=132), with characteristics described in *Table 1*. The two groups significantly differed in smoking and alcohol consumption history, BMI, ALB, TG, HDL-C, LDL-C, GGT, and SUA. BMI (mean, 24.54 *vs.* 23.96), TG (median, 1.09 *vs.* 0.92), and LDL-C (median, 2.66 *vs.* 2.49) were lower in older group than in younger group (P=0.048, <0.001, and 0.013, respectively). Whereas HDL-C was higher in the aged group than in the younger group (median, 1.24 *vs.* 1.15; P=0.009). This phenomenon may be related to lipid metabolism change or lipid-lowering medication in older men. Because of the obvious differences in these subgroups (≤75 *vs.* >75 years), the following analysis was conducted in total and subgroups.

Association between PV and IBIL

TC was dropped from the analysis due to collinearity with LDL-C. The univariable linear regression analysis (*Table 2*) revealed that PV positively correlated with age, BMI, TC, and LDL-C in the younger group (β=0.151, 0.121, 0.102, 0.118, respectively). While in the older group, PV positively correlated with TG, TC, and LDL-C (β=0.215, 0.220, 0.231, respectively). Then we focus on the IBIL-PV relationship. The Spearman scatterplot (Figure S2) did not show significant correlation between IBIL and PV in total (r=–0.039, P=0.294), younger group (r=–0.081, P=0.071), and older group (r=0.139, P=0.112). Similarly, the unadjusted model (crude model) of linear regression analyses did not show significant correlation between IBIL and PV in total (β=–0.46, P=0.22), younger group (β=–0.77, P=0.06), and older group (β=1.13, P=0.22)

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Table 1 The baseline clinical characteristics in age subgroups

Factors	Total (n=722)	Age ≤75 years (n=590)	Age >75 years (n=132)	P
Hypertension (%)				0.095
No	62.41	63.84	56.06	
Yes	37.59	36.16	43.94	
Diabetes (%)				0.751
No	83.93	83.73	84.85	
Yes	16.07	16.27	15.15	
Smoking (%)				0.04*
Never	63.85	61.86	72.73	
Current	5.13	5.76	2.27	
Former	31.03	32.37	25.00	
Alcohol (%)				0.033*
Never	65.24	63.05	75.00	
Current	4.43	4.75	3.03	
Former	30.33	32.20	21.97	
BMI[#] (kg/m^2)	24.43±3.02	24.54±3.01	23.96±3.02	0.048
PV (mL)	62.84 (42.73, 86.79)	63.28 (41.86, 86.42)	59.49 (47.29, 88.40)	0.8
ALB (g/L)	40.70 (38.80, 42.70)	41.10 (39.20, 42.90)	39.40 (37.50, 41.10)	<0.001*
DBIL (μmol/L)	3.50 (2.70, 4.60)	3.50 (2.70, 4.60)	3.60 (2.70, 5.10)	0.314
IBIL (μmol/L)	7.80 (6.00, 10.40)	7.90 (6.00, 10.50)	7.60 (6.00, 9.40)	0.35
TG[#] (mmol/L)	1.05 (0.79, 1.45)	1.09 (0.82, 1.48)	0.92 (0.71, 1.28)	<0.001*
TC[#] (mmol/L)	4.08 (3.54, 4.63)	4.09 (3.58, 4.64)	3.97 (3.46, 4.55)	0.088
HDL-C (mmol/L)	1.15 (0.96, 1.37)	1.15 (0.95, 1.35)	1.24 (1.00, 1.46)	0.009*
LDL-C (mmol/L)	2.62 (2.12, 3.10)	2.66 (2.16, 3.12)	2.49 (1.98, 2.93)	0.013*
GGT[#] (U/L)	19.60 (14.80, 26.30)	19.80 (15.00, 26.60)	18.60 (14.30, 24.80)	0.034*
GLU[#] (mmol/L)	4.86 (4.50, 5.42)	4.86 (4.50, 5.40)	4.85 (4.49, 5.45)	0.967
SUA (μmol/L)	322.7 (278.6, 376.4)	319.0 (275.4, 372.7)	344.2 (298.3, 391.3)	0.005*

[#] indicates the number of missing values (BMI: 2, TG: 15, TC: 2, GGT: 3, GLU: 1); *P<0.05. BMI, body mass index; PV, prostate volume; ALB, albumin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; GGT, gamma-glutamyltransferase; GLU, glucose; SUA, serum uric acid.

(*Table 3*). However, after adjusting for age, BMI, and TG (model 1) or adjusting for age, BMI, TG, and LDL-C (model 2), we observed significant inverse IBIL-PV relationship in the age ≤75 years group (model 1: β=−0.82, P=0.04; model 2: β=−1.01, P=0.01) (*Table 3*). By contrast, the DBIL-PV relationship was significant in the crude model and model 1 but not significant in model 2. The results were similar to those in multiple stepwise regression analysis of variables with PV (Table S1). In addition, there was a statistically significant inverse trend between the three intervals of IBIL and PV in the younger group after adjusting for age, BMI, and LDL-C (*Table 4*). In younger group, compared to the subgroup with IBIL <6 μmol/L, there were significant negative associations of PV in the subgroup with 6.1 μmol/L ≤ IBIL ≤12.4 μmol/L (adjusted β=−7.123; 95% CI: −13.783, −0.463) and subgroup with

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Table 2 univariable linear regression analysis of PV and other variables

Variables	Total		Age ≤75 years		Age >75 years	
	β	P	β	P	β	P
Hypertension						
No	Ref.		Ref.		Ref.	
Yes	0.016	0.669	0.002	0.963	0.093	0.288
Diabetes						
No	Ref.		Ref.		Ref.	
Yes	−0.029	0.438	−0.035	0.399	<0.001	0.998
Smoking						
Never	Ref.		Ref.		Ref.	
Current	−0.053	0.158	−0.065	0.118	0.044	0.616
Former	−0.059	0.116	−0.046	0.275	−0.127	0.149
Drinking						
Never	Ref.		Ref.		Ref.	
Current	−0.028	0.464	−0.03	0.47	−0.009	0.923
Former	0.01	0.792	0.016	0.706	−0.016	0.854
Age (years)	0.113	0.002*	0.151	<0.001*	0.141	0.106
BMI (kg/m^2)	0.096	0.010*	0.121	0.003*	−0.015	0.861
ALB (g/L)	0.030	0.419	−0.207	0.513	0.051	0.540
DBIL (μmol/L)	−0.068	0.068	−0.085	0.040*	0.004	0.960
IBIL (μmol/L)	−0.046	0.216	−0.077	0.062	0.109	0.215
GLU (mmol/L)	−0.017	0.651	−0.016	0.695	−0.020	0.817
TG (mmol/L)	0.062	0.094	0.037	0.369	0.215	0.013*
TC (mmol/L)	0.121	0.001*	0.102	0.013*	0.220	0.011*
HDL-C (mmol/L)	−0.070	0.062	−0.065	0.116	−0.098	0.264
LDL-C (mmol/L)	0.135	<0.001*	0.118	0.004*	0.231	0.008*
GGT (U/L)	0.007	0.850	−0.007	0.870	0.114	0.195
SUA (μmol/L)	0.034	0.357	0.030	0.465	0.053	0.543

*P<0.05. PV, prostate volume; BMI, body mass index; ALB, albumin; DBIL, direct bilirubin; IBIL, indirect bilirubin; GLU, glucose; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; GGT, gamma-glutamyltransferase; SUA, serum uric acid.

IBIL >12.4 μmol/L (adjusted β=−11.187; 95% CI: −21.158, −1.216). The overall adjusted β was −5.944 (95% CI: −10.733, −1.154), and the P value for the trend was 0.015 (*Table 4*). In comparison, we failed to identify significant relationship of IBIL and PV in the group >75 years (*Tables 3*,*4* and Table S1).

Sensitivity analysis

In sensitivity analysis based on BMI and LDL-C stratification, after adjusting for age, BMI and LDL-C, the significantly negative IBIL-PV relationship only existed in men with normal BMI (adjusted β=−1.328; 95% CI:

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Table 3 Associations of DBIL and IBIL with PV in total and subgroups

Groups	Crude model		Adjusted model 1		Adjusted model 2	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
Total (n=722)						
DBIL	−1.48 (−3.07, 0.11)	0.07	1.51 (−3.01, 0.09)	0.06	−0.77 (−2.41, 0.87)	0.36
IBIL	−0.46 (−1.19, 0.27)	0.22	−0.52 (−1.25, 0.21)	0.16	−0.72 (−1.442, 0.012)	0.05
Age ≤75 years (n=590)						
DBIL	−1.86 (−3.64, −0.09)	0.04*	−1.96 (−3.72, −0.19)	0.03*	−1.34 (−3.16, 0.48)	0.15
IBIL	−0.77 (−1.57, 0.04)	0.06	−0.82 (−1.62, −0.03)	0.04*	−1.01 (−1.81, −0.22)	0.01*
Age >75 years (n=132)						
DBIL	0.09 (−3.50, 3.68)	0.96	1.56 (−2.08, 5.21)	0.4	2.72 (−0.97, 6.41)	0.15
IBIL	1.13 (−0.65, 2.91)	0.22	1.56 (−0.19, 3.32)	0.08	1.36 (−0.38, 3.11)	0.13

*P<0.05. The crude model was unadjusted. Model 1 was adjusted for age, BMI, and TG; and model 2 was for age, BMI, TG, and LDL-C. DBIL, direct bilirubin; IBIL, indirect bilirubin; PV, prostate volume; CI, confidence interval; BMI, body mass index; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol.

Table 4 Linear trend test of serum IBIL cutoffs and PV in total and subgroups

IBIL (μmol/L)	N	Crude model		Adjusted model	
		β (95% CI)	P	β (95% CI)	P
Total					
≤6	173	Ref.		Ref.	
6.1–12.4	465	−1.06 (−7.161, 5.041)	0.733	−2.757 (−8.772, 3.258)	0.369
>12.4	84	−4.483 (−13.594, 4.627)	0.335	−7.465 (−16.500, 1.571)	0.105
P for trend	722	−1.966 (−6.332, 2.399)	0.377	−3.505 (−7.839, 0.829)	0.113
Age ≤75 years					
≤6	140	Ref.		Ref.	
6.1–12.4	381	−5.655 (−12.449, 1.139)	0.103	−7.123 (−13.783, −0.463)	0.036*
>12.4	69	−8.735 (−18.847, 1.377)	0.09	−11.187 (−21.158, −1.216)	0.028*
P for trend	590	−4.662 (−9.514, 0.190)	0.06	−5.944 (−10.733, −1.154)	0.015*
Age >75 years					
≤6	33	Ref.		Ref.	
6.1–12.4	84	18.801 (5.326, 32.277)	0.006*	16.528 (3.221, 29.835)	0.015*
>12.4	15	13.882 (−6.543, 34.308)	0.183	12.732 (−7.642, 33.107)	0.221
P for trend	132	9.924 (0.113, 19.735)	0.047*	8.978 (−0.770, 18.726)	0.071

*P<0.05. The crude model was unadjusted. The adjusted model was adjusted for age, BMI, and LDL-C. IBIL, indirect bilirubin; PV, prostate volume; CI, confidence interval; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol.

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Table 5 Stratified analyses for PV-IBIL relationship in BMI and LDL-C subgroups

Groups	N	Crude model		Adjusted model	
		β (95% CI)	P	β (95% CI)	P
Total	590	−0.766 (−1.568, 0.036)	0.061	−0.998 (−1.791, −0.206)	0.014*
BMI (kg/m^2)					
Normal	247	−1.177 (−2.278, −0.076)	0.036*	−1.328 (−2.467, −0.190)	0.022*
Overweight	273	−1.169 (−2.433, 0.094)	0.07	−1.296 (−2.519, −0.074)	0.038*
Obesity	70	2.209 (−0.472, 4.530)	0.112	1.788 (−0.726, 4.301)	0.163
LDL-C (mmol/L)					
<3.4	498	−0.758 (−1.613, 0.097)	0.082	−1.017 (−1.869, −0.164)	0.019*
≥3.4	92	−1.117 (−3.372, 1.139)	0.332	−1.26 (−3.455, 0.934)	0.26

*P<0.05. The crude model was unadjusted, whereas the second model was adjusted for age, BMI, and LDL-C. PV, prostate volume; IBIL, indirect bilirubin; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; CI, confidence interval.

−2.467, −0.190; P=0.022) and overweight BMI (adjusted β=−1.296; 95% CI: −2.519, −0.074; P=0.038) (*Table 5*). Similarly, negative IBIL-PV relationship was only detected in subgroup with normal LDL-C (adjusted β=−1.017; 95% CI: −1.869, −0.164; P=0.019). Comparatively, for patients ≤75 years, no negative correlation between PV and IBIL was found in obesity men (adjusted β=1.788; 95% CI: −0.726, 4.301; P=0.163) and men with high level of LDL-C (adjusted β=−1.26; 95% CI: −3.455, 0.934; P=0.26) in the full adjusted model (*Table 5*).

Moreover, to identify the influence of outliers, we conducted a sensitivity analysis including all 730 subjects. The inverse IBIL-PV relationship in men ≤75 did not change (Table S2).

Discussion

Due to its antioxidant effects, serum bilirubin protects against multiple diseases, including CVD, cancer, diabetes, and MetS (12). TBIL has two types: DBIL and IBIL. Most previous literature studied the effects of TBIL. In the present study, we observed that only IBIL levels were inversely correlated with PV in patients with BPH ≤75 years after adjusting for age, BMI, and LDL-C. Based on the available literature, there does not seem to be a previous report on the relationship between IBIL and BPH. However, this is not surprising given that IBIL exerts antioxidant activity and anti-inflammatory effects (9,18). Of note, the impact of IBIL was not significant in the unadjusted model, while prominent after adjusting for confounders such as age, BMI, and LDL-C. In the multivariable linear regression model, these cofounders had a more dramatic effect on PV than IBIL (*Table 2*, Table S1). Therefore, we hypothesized that IBIL indirectly influences BPH via attenuating OS related to age, obesity, and lipid peroxidation. Here the discussion will be presented on the relationship between IBIL and three confounders and their possible mechanisms for BPH.

It is widely accepted that age is associated with the incidence and progression of BPH (19). OS contributes to aging and aging-related disease, including BPH (20-22). Due to its antioxidant properties, bilirubin affects aging and aging-related diseases (23,24). An experimental animal study indicated that mildly elevated serum bilirubin is generally associated with the alleviation of OS, reduced inflammatory status, and fewer signs of cellular senescence (25). In this study, we also observed that the inverse PV-IBIL relation existed in men ≤75 years. However, aging progression leads to more severe OS (26), exceeding the antioxidant capacity of serum IBIL, which may be the reason for the non-significant effect of IBIL in men >75.

Meanwhile, we found that BMI was positively associated with PV in total and subgroups in addition to age. And BMI was an important confounding factor in the PV-IBIL relationship (*Tables 3-5*). This finding is consistent with previous reports, indicating that higher BMI is an independent risk factor for BPH (27-29). Meanwhile, OS levels increased when BMI increased (30). By stratified analysis, we observed an inverse PV-IBIL relationship in normal and overweight men but insignificant in obese individuals. Compared with normal conditions, obese men have a higher level of OS (31). Maybe the antioxidant

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capacity of IBIL cannot counteract severe OS in obese individuals. Perhaps, that might explain why the PV-IBIL relationship was not significant in obese men.

LDL-C was positively correlated with PV in all BPH patients. Nandeesha *et al.* demonstrated that the LDL-C level was higher in patients with symptomatic BPH than in controls (32). Previous studies have also observed more elevated lipoprotein peroxidation activity (33) or oxidized LDL (ox-LDL) levels (34) in the blood of patients with BPH compared to healthy control patients. Ox-LDL is a crucial contributor to the pathogenesis of atherosclerosis (35). Haga *et al.* demonstrated that the local atherosclerosis of the artery in the prostate is significantly associated with prostate size (36). In atherogenic conditions, ox-LDL plays a vital role in the upregulation of prostatic cell proliferation by activating the Rho/Akt/p27 (Kip1) pathways (37).

On the other hand, it was previously revealed that both IBIL and DBIL alone could protect human LDLs against oxidation. However, on a per mole basis, IBIL has more potent anti-oxidation properties for LDLs than DBIL (38). This difference is perhaps one reason that only IBIL (not DBIL) showed a negative relationship with PV in our study (*Table 3*) after adjusting for confounders. It is reasonable to speculate that IBIL exerts a protective effect by alleviating LDL-C oxidation and atherosclerosis (39) in BPH men ≤75 years. In contrast, the IBIL-PV relationship was not apparent in the group with LDL-C exceeding the normal range (*Table 5*). Similar to the analysis above, one possible explanation is that more LDL-C was transformed to ox-LDL, and IBIL cannot compensate for the more severe OS.

Although this study has made some intriguing discoveries, its limitations should be acknowledged. First, considering the high prevalence of BPH, the sample size is relatively insufficient. As this was a single-center study in China, the current findings cannot be extrapolated to other ethnic groups. Subjects from multiple centers and different ethnicities should be included in future studies. Second, this article's potential factors associated with PV are not comprehensive. For example, these patients did not test serum steroid hormones related to prostate status. Third, as our study was retrospective, additional epidemiological data, including prospective studies, are required to confirm the PV-IBIL relationship. Moreover, future mechanism studies are warranted to elucidate the underlying mechanism comprehensively.

In conclusion, we observed for the first time that IBIL was negatively associated with PV after adjusting for age, BMI, and LDL-C in non-obese BPH patients ≤75 years with normal LDL-C levels. IBIL may attenuate BPH progression by mitigating OS related to aging and LDL-C oxidation. Serum IBIL appears to be a potential candidate biomarker to predict and modulate BPH processes before age 75. This study provides a reference for the early monitoring of BPH. We expect our study to help provide new ideas for the intervention and clinical treatment of BPH.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-3997/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3997/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Chinese PLA General Hospital (No. S2021-364-01), and individual consent for this retrospective analysis was waived.

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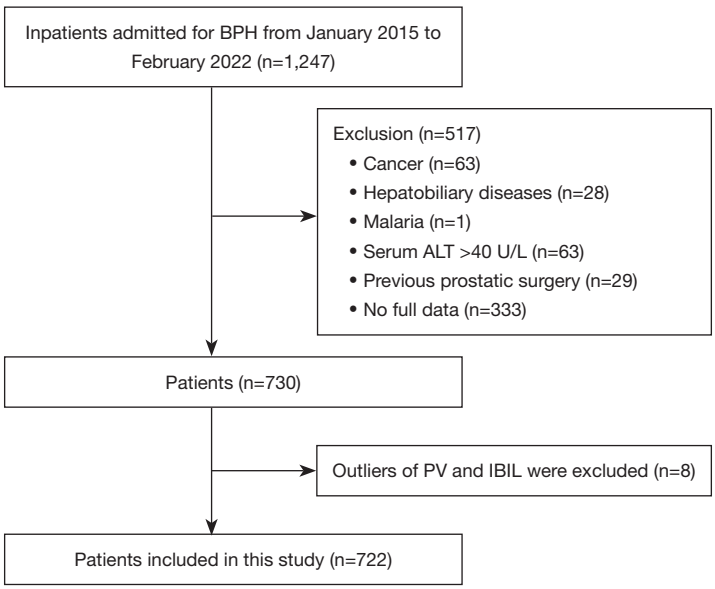

Figure S1 Flow chart of the inclusion/exclusion process. BPH, benign prostatic hyperplasia; ALT, alanine aminotransferase; PV, prostate volume; IBIL, indirect bilirubin.

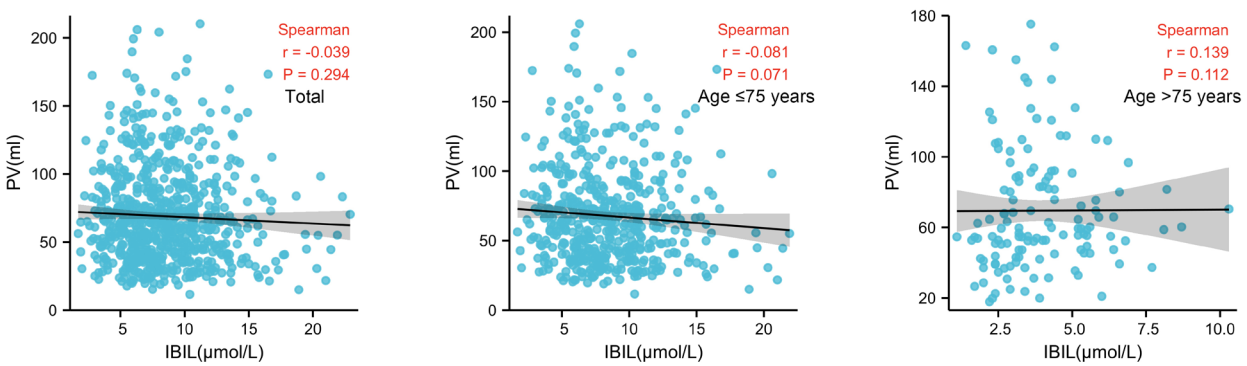

Figure S2 The scatter plot of IBIL-PV data (by Spearman correlation). PV, prostate volume; IBIL, indirect bilirubin.

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Table S1 Multiple stepwise regression analysis of variables with PV in the entire population and subgroups

Age subgroups	B (adjusted)	β (adjusted)	95% CI (adjusted)		P (adjusted)
			Upper	Lower	
Total					
Age	0.614	0.137	0.289	0.938	<0.001*
BMI	1.13	0.098	0.299	1.962	0.008*
LDL-C	6.74	0.145	3.379	10.1	<0.001*
Age ≤75 years					
Age	0.937	0.16	0.474	1.399	<0.001*
BMI	1.509	0.129	0.589	2.428	0.001*
LDL-C	6.45	0.139	2.747	10.153	0.001*
IBIL	−0.998	−0.1	−1.792	−0.204	0.014*
Age >75 years					
LDL-C	11.119	0.231	2.993	19.246	0.008*

*P<0.05. PV, prostate volume; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; IBIL, indirect bilirubin; CI, confidence interval.

Table S2 Associations of DBIL and IBIL with PV in total and subgroups (outlier samples were not removed)

Groups	Crude model		Adjusted model 1		Adjusted model 2	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
Total (n=730)						
DBIL	−1.406 (−2.985, 0.172)	0.081	−1.36 (−2.954, 0.233)	0.094	−0.687 (−2.319, 0.946)	0.41
IBIL	−0.429 (−1.155, 0.297)	0.246	−0.442 (−1.166, 0.282)	0.232	−0.595 (−1.316, 0.127)	0.106
Age ≤75 years (n=594)						
DBIL	−1.618 (−3.322, 0.087)	0.063	−1.698 (−3.408, 0.012)	0.052	−1.144 (−2.898, 0.611)	0.201
IBIL	−0.643 (−1.405, 0.120)	0.099	−0.70 (−1.465, 0.051)	0.068	−0.863 (−1.621, −0.104)	0.026*
Age >75 years (n=136)						
DBIL	−0.853 (−4.867, 3.160)	0.677	0.578 (−3.620, 4.775)	0.787	1.788 (−2.503, 6.079)	0.414
IBIL	0.671 (−1.384, 2.726)	0.522	1.199 (−0.903, 3.301)	0.263	1.076 (−1.008, 3.161)	0.311

Dependent variable: PV. *P<0.05. The crude model was unadjusted. Model 1 was adjusted for age, BMI, and TG; model 2 was adjusted for age, BMI, TG, and LDL-C. DBIL, direct bilirubin; IBIL, indirect bilirubin; PV, prostate volume; CI, confidence interval; BMI, body mass index; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol.

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