RESEARCH ARTICLE

Serum bilirubin and the risk of rheumatoid arthritis

Du Juping^{1,#} | Yuan Yuan^{1,#} | Chen Shiyong¹ | Li Jun¹ | Zhou Xiuxiu² | Ying Haijian³ | Shi Jianfeng³ | Shen Bo^{1,3}¹Department of Clinical Laboratory, Taizhou Hospital of Zhejiang Province, Taizhou, Zhejiang Province, China²Department of Clinical Laboratory, Linhai City of Traditional Chinese Hospital, Linhai, Zhejiang Province, China³Department of Clinical Laboratory, Taizhou Hospital of Zhejiang Province, Affiliated Hospital of Wenzhou Medical College, Taizhou, Zhejiang Province, China

Correspondence

Shen Bo, Department of Clinical Laboratory, Taizhou Hospital of Zhejiang Province, Linhai, Zhejiang, China.
Email: 1282437698@qq.com**Background:** Oxidative stress and immune imbalance play an important role in the pathogenesis of rheumatoid arthritis (RA). Bilirubin is a powerful antioxidant and also regarded as immunomodulator. Increased evidence shows that bilirubin should be a protective factor for autoimmune disease. However, the relationship between bilirubin and RA remain unclear.**Methods:** We analyzed serum bilirubin levels and other laboratory and clinical data in 130 RA patients (35 patients without any complications), 81 osteoarthritis (OA) patients and 96 healthy controls.**Results:** Binary logistic regression adjusted by age and gender revealed that the levels of serum total, indirect bilirubin were significantly lower in RA patients, when compared with healthy controls ($P=.015$, $OR=0.767$, 95% $CI=0.619-0.951$; $P=.010$, $OR=0.664$, 95% $CI=0.487-0.906$, respectively) or OA patients ($P=.000$, $OR=0.763$, 95% $CI=0.661-0.882$; $P=.000$, $OR=0.656$, 95% $CI=0.532-0.808$, respectively). A reduced trend of levels of bilirubin has been detected along with increased disease activity, despite with no significance ($P>.05$). Spearman rank test further demonstrated that IgG and ESR were negative associated with total, indirect bilirubin, and albumin, prealbumin, APOA, HDL-C were positively associated with bilirubin.**Conclusions:** In conclusion, the levels of serum bilirubins were decreased in RA, and decreased levels could be associated with IgG, albumin and inflammatory marker ESR.

KEYWORDS

bilirubin, oxidative stress, rheumatoid arthritis

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease with unknown etiology and characterized by the infiltrating inflammatory cells and synovial fibroblasts in the joints, leading to irreversible destruction of cartilage and bone. It has been established that oxidative stress has distinct contribution to the RA inflammation and tissue damage.¹⁻³ During inflammation, immune activated cells in the inflamed RA synovium could generate mass of reactive oxygen species (ROS) via the NADPH oxidase system.⁴ Excess ROS and ROS-oxidized production initial the proinflammatory responses in RA along with the damage to the lipids, proteins, and DNA,⁵ which leading to the production of autoantibodies. Overexpression ROS has been detected in the blood and

synovial fluid of RA patients. Of these, 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a biomarker for oxidized DNA and ROS, has been found correlated with RA disease activity.⁶ Accumulating evidence show that antioxidants might be an effective therapy to alleviate the illness for RA.^{7,8} Thus, oxidative stress and inflammation might contribute to the major pathogenesis of RA in the disease initial and progression.

Bilirubin is a powerful antioxidant to the ability of scavenging ROS and also has anti-inflammatory and immune suppressive activities by inhibiting inflammatory cell proliferation, inducing reactive inflammatory cell apoptosis, reducing pro-inflammatory cytokines, and down-regulating the expression of MHC-II.^{9,10} The antioxidant capacity of bilirubin is stronger than other antioxidants, such as ascorbic acid, catalase, and vitamin E. Lower serum concentration of bilirubin has been demonstrated in various chronic inflammatory diseases, including systemic lupus erythematosus, polymyositis, hypertension, and coronary

#Du Juping and Yuan Yuan equally contributed to this study as first coauthors.

artery calcification.¹¹⁻¹³ However, to our knowledge, the role of serum bilirubin with RA has not been sufficiently investigated.

Hence, the aim of this retrospective cross-sectional study was to examine the role of decreased serum bilirubin as a risk factor for RA and the correlation with clinical and laboratory characteristics of RA.

2 | MATERIALS AND METHODS

2.1 | Subjects

One hundred fifty-two consecutive RA patients who fulfilled the 2010 criteria of American College of Rheumatology/European League Against Rheumatism and admitted to the Taizhou Hospital from January 2012 to December 2015 were enrolled in this retrospective study. Twenty-two patients with hepatobiliary, gallbladder, or hemolytic diseases that could have a change on the bilirubin level were excluded. Laboratory parameters and basic clinical characteristics including blood pressure, BMI, medication, smoking/drinking habits, disease duration, morning stiffness and history of hypertension, cardiovascular disease, diabetes, and pulmonary diseases were recorded from medical records. Based on the 28-joint disease activity score (DAS28), RA patients were divided into three groups: low activity group (DAS28<3.2), moderate activity group (3.2≤DAS28<5.1), and high activity group (DAS28≥5.1). In addition, a group of 81 hospitalized osteoarthritis (OA) patients met the ACR classification criteria of OA were also recruited as disease control group, since OA patients always present the same clinical symptoms with RA. Ninety-six healthy volunteers were randomly selected in this study. All the OA patients and healthy control were confirmed to have no family and personal history of autoimmune disease, cancers, liver disease, renal diseases, cardiovascular disease, or other inflammatory diseases. Since complications might be the important confounding factors that influence the levels of bilirubins, only 35 RA patients without any other diseases were included for comparing with OA patients and healthy controls (HC). The study was approved by the institutional review board of Ethics Committee of the Taizhou Hospital of Zhejiang province, and all the participants signed the written informed consent.

2.2 | Data analysis

Since all continuous variables were non-normal distribution checked by Kolmogorov-Smirnov test, data were expressed as median (25th-75th percentiles). Binary logistic regression analysis with adjusted by age and gender was carried out to compare the differences in the laboratory data and clinical characteristics between RA patients and OA/HC group. Kruskal-Wallis *H* nonparametric test was used to compare three groups. The serum bilirubin was divided into two groups with the median as the cutoff. Then, univariate and multivariate logistic regressions were performed to determine the independent predictors to the change of serum bilirubin. The correlation between bilirubin and other laboratory or clinical data was assessed using Spearman's rank test. Statistical analysis was conducted by SPSS 22.0 software (SPSS Inc., Chicago, IL, USA), and scatter plot was performed using GraphPad Prism (GraphPad Prism Software Inc., San Diego, CA, USA). A *P* value <.05 was considered statistically significant.

3 | RESULTS

3.1 | The serum bilirubin level in RA patients and OA patients or healthy controls

Thirty-five RA patients without any complications (female 77.1%, median age 59.0) were included in comparison with 96 healthy controls (female 45.8%, median age 40.0) or 81 OA patients (female 42.0%, median age 56.0). The median disease duration in RA was 5 years. We compared serum total bilirubin, direct bilirubin, indirect bilirubin, LDL-C, HDL-C, ESR, CRP, albumin, A/G, NEU, LYM, and BMI levels of RA patients with OA and healthy controls by binary logistic regression with adjustment of age and gender (Table 1). In the RA group, median total bilirubin was 7.5 (6.2-9.0) μmol/L. This was significantly lower than OA patients (median 10.6 [8.1-13.5]) and HC group (median 11.2 [9.5-13.5]), *P*<.05. In addition, indirect bilirubin was also significantly lower in RA patients than OA (*P*<.001, OR=0.656, 95% CI=0.532-0.808) and HC (*P*<.010, OR=0.664, 95% CI=0.487-0.906), whereas serum direct bilirubin had marginal significance between RA and HC groups (*P*=.093, OR=0.644, 95% CI=0.386-1.076) but significant difference between RA and OA patients (*P*=.035, OR=0.678, 95% CI=0.473-0.973, respectively; Table 1 and Figure 1). Moreover, there were lower median levels of albumin and A/G, but higher ESR and NEU in RA patients than in OA patients and HC group (*P*<.001). Remarkably, 75th percentiles of albumin and A/G in RA group were much lower than these in OA and HC groups.

3.2 | Association of serum bilirubin levels with RA disease activity

Based on DAS28, RA patients were classified into three groups. One patient due to lack of clinical data was excluded, and 129 RA patients remained for further analysis. When the three groups were compared, there were no significant differences detected for age, gender, smoke status, alcohol consumption, and duration (Table 2). Then, increased disease activity was accompanied by a decreasing trend in serum total bilirubin (median, 9.80 vs 7.45 vs 7.45 μmol/L), indirect bilirubin (median, 6.70 vs 4.65 vs 4.90 μmol/L), and direct bilirubin (median, 4.00 vs 2.50 vs 2.40 μmol/L), but without significance detected. Moreover, larger proportion of morning stiffness was detected in high activity group. Then, we combined moderate activity RA patients with high activity group together (DAS28≥3.2) and observed that the median indirect bilirubin in DAS28<3.2 group (median 6.70 μmol/L) was significantly higher than that in DAS28≥3.2 RA patients (median 4.90 μmol/L; *P*=.03), data not shown. However, we still failed to validate a similarly significant association in total bilirubin (*P*=.053) and direct bilirubin (0.269).

3.3 | Association of serum bilirubin levels with laboratory and clinical data in RA patients

The median was determined to the cutoff value of serum bilirubin. We analyzed the association of total bilirubin (≤7.5/>7.5), direct bilirubin (≤2.5/>2.5), and indirect bilirubin (≤5.0/>5.0) with laboratory and clinical variables by univariate logistic regression (Table 3). It was

TABLE 1 Clinical and laboratory characteristics in rheumatoid arthritis (RA) patients, osteoarthritis (OA) patients, and healthy controls

	RA patients (35 cases)	Healthy control (96 cases)			OA patients (81 cases)		
	Median (IQR)	Median (IQR)	P_{ad}	OR_{ad} (95% CI)	Median (IQR)	P_{ad}	OR_{ad} (95% CI)
Age (y)	59.0 (51.0-67.0)	40.0 (30.3-49.0)	.000	1.201 (1.121-1.287)	56.0 (45.0-64.0)	.000	1.082 (1.045-1.120)
Female	27 (77.1)	44 (45.8)	.001	0.100 (0.026-0.381)	34 (42.0)	.000	1.068 (1.038-1.100)
Total bilirubin ($\mu\text{mol/L}$)	7.5 (6.2-9.0)	11.2 (9.5-13.5)	.015	0.767 (0.619-0.951)	10.6 (8.1-13.5)	.000	0.763 (0.661-0.882)
Direct bilirubin ($\mu\text{mol/L}$)	2.4 (1.8-3.2)	4.2 (3.5-4.8)	.093	0.644 (0.386-1.076)	3.3 (2.5-4.4)	.035	0.678 (0.473-0.973)
Indirect bilirubin ($\mu\text{mol/L}$)	4.9 (3.9-6.6)	7.3 (5.7-8.7)	.010	0.664 (0.487-0.906)	7.2 (5.6-9.7)	.000	0.656 (0.532-0.808)
LDL-C (mmol/L)	2.0 (1.6-3.1)	2.2 (2.0-2.7)	.702	0.875 (0.441-1.736)	1.3 (1.2-1.6)	.016	0.532 (0.318-0.890)
HDL-C (mmol/L)	1.2 (0.9-1.4)	1.3 (1.2-1.6)	.115	0.211 (0.030-1.463)	2.7 (1.9-3.2)	.006	0.125 (0.028-0.552)
ESR (mm/h)	76.0 (41.0-104.0)	9.0 (5.0-12.0)	.014	2.252 (1.182-4.293)	44.0 (39.5-46.1)	.000	1.089 (1.057-1.121)
Albumin (g/L)	36.7 (32.6-39.7)	47.3 (45.4-48.8)	.001	0.430 (0.260-0.711)	1.6 (1.4-1.8)	.000	0.712 (0.625-0.812)
NEU ($10^9/\text{L}$)	5.0 (3.7-6.8)	3.1 (2.7-3.8)	.001	2.675 (1.490-4.805)	1.9 (1.5-2.3)	.000	1.541 (1.249-1.903)
LYM ($10^9/\text{L}$)	2.0 (1.3-2.5)	2.1 (1.7-2.4)	.429	1.328 (0.657-2.684)	10.0 (4.0-21.0)	.269	1.384 (0.778-2.463)
BMI	21.4 (18.2-24.2)	23.0 (21.0-24.6)	.013	0.742 (0.586-0.938)			

The analysis was adjusted by gender and age with binary logistic regression.

FIGURE 1 Analysis of serum bilirubin levels of rheumatoid arthritis patients ($n=35$), osteoarthritis patients ($n=81$), and healthy controls ($n=96$). (a) Total bilirubin, (b) direct bilirubin, and (c) indirect bilirubin. *** $P<.001$

found that higher total bilirubin was associated with higher A/G and prealbumin. Those with higher indirect bilirubin had higher A/G, albumin, CHE, and TBA, but lower prealbumin. Interestingly, those with higher IgG level had lower total bilirubin, direct bilirubin, and indirect bilirubin ($P<.05$). Unfortunately, we failed to detect the similar association in RF and inflammatory markers (such as CRP and ESR).

Then, univariate variables with $P<.1$ were included in the multivariate logistic regression (Table 4). However, only prealbumin remained as an independent predictor for serum total bilirubin ($P=.033$, $OR=1.166$, 95% $CI=1.013$ - 1.343) and indirect bilirubin ($P=.018$, $OR=1.212$, 95% $CI=1.034$ - 1.421). Even more puzzling was that those with higher CRP levels had higher direct bilirubin, despite the OR value was really small ($P=.048$, $OR=1.010$, 95% $CI=1.000$ - 1.019).

As shown in Table 5, the IgG levels of RA patients inversely correlated with serum total bilirubin, direct bilirubin, and indirect bilirubin ($r=-.338$, $P=.001$; $r=-.230$, $P=.027$; $r=-.337$, $P=.001$, respectively). In addition, both total bilirubin and indirect bilirubin levels positively correlated with albumin, A/G, prealbumin, APOA, APOB, and HDL-C, but inversely correlated with ESR ($P<.05$).

4 | DISCUSSION

In this study, we demonstrated that serum bilirubin levels (including total, indirect, and direct bilirubins) were significantly lower in RA patients than osteoarthritis and healthy controls, especially in RA patients with high disease activity individuals. Furthermore, the relationship between serum bilirubin levels and some laboratory and clinical variables has been examined in RA patients.

Early in 1929, Philip Hench et al. made an interesting observation that the symptoms of rheumatoid arthritis relieved with the onset of jaundice or hyperbilirubinemia, indicating that high bilirubin might have the protect ability against the inflammatory diseases.^{14,15} Also, in a large epidemiologic study, serum total bilirubin has been confirmed to be associated with a decreased risk of rheumatoid arthritis.¹⁶ These results support our findings that serum bilirubin (including total, indirect, and direct bilirubin) has a significantly decreased level in RA patients relative to healthy controls, even to the osteoarthritis patients. Moreover, the decreased trend of serum bilirubin levels accompanied by increased the disease activity have been detected, albeit only

Group	DAS28<3.2	DAS28=3.2-5.1	DAS28>5.1
	Median (IQR)	Median (IQR)	Median (IQR)
Age (y)	59.0 (46.0-69.5)	58.5 (54.0-67.0)	65.0 (59.0-70.5)
Male	2 (22.2)	19 (32.8)	19 (30.6)
Smoke	1 (11.1)	9 (15.5)	16 (25.8)
Alcohol consumption	1 (11.1)	5 (8.6)	7 (11.3)
Morning stiffness	7 (77.8)	30 (51.7)	52 (83.9)
Duration (y)	10.0 (3.0-23.5)	7.0 (2.0-20.0)	6.0 (1.0-15.3)
Charlson	3.0 (1.0-3.5)	2.0 (1.0-3.0)	3.0 (2.0-4.0)
Total bilirubin ($\mu\text{mol/L}$)	9.80 (7.05-14.20)	7.45 (5.30-10.65)	7.45 (6.08-9.00)
Direct bilirubin ($\mu\text{mol/L}$)	4.00 (1.80-5.35)	2.50 (1.50-3.53)	2.40 (1.88-3.20)
Indirect bilirubin ($\mu\text{mol/L}$)	6.70 (5.00-8.85)	4.65 (3.30-7.40)	4.90 (3.70-5.90)

TABLE 2 The association between serum bilirubin levels and disease activity

TABLE 3 Association between serum bilirubin levels and clinical and laboratory characteristics in rheumatoid arthritis patients

	Total bilirubin ($\leq 7.5 / > 7.5 \mu\text{mol/L}$)		Direct bilirubin ($\leq 2.5 / > 2.5 \mu\text{mol/L}$)		Indirect bilirubin ($\leq 5.0 / > 5.0 \mu\text{mol/L}$)	
	OR	P	OR	P	OR	P
Age (y)	0.988 (0.954-1.023)	.488	0.972 (0.938-1.006)	.108	0.997 (0.963-1.032)	.868
Male	1.397 (0.661-2.951)	.381	1.045 (0.496-2.202)	.907	1.156 (0.548-2.435)	.704
Charlson score	0.996 (0.796-1.247)	.973	0.896 (0.713-1.125)	.345	1.027 (0.820-1.285)	.819
Albumin (g/L)	1.055 (0.991-1.124)	.093	0.998 (0.930-1.050)	.695	1.088 (1.019-1.162)	.012
TBA ($\mu\text{mol/L}$)	1.071 (0.981-1.170)	.126	1.045 (0.970-1.125)	.244	1.184 (1.051-1.334)	.005
A/G	5.595 (1.443-21.692)	.013	2.392 (0.653-8.755)	.188	12.391 (2.937-52.270)	.001
CHE (KU/L)	1.205 (0.996-1.458)	.055	0.871 (0.724-1.050)	.147	1.244 (1.025-1.510)	.027
Ada (U/L)	0.994 (0.896-1.103)	.915	1.022 (0.921-1.134)	.686	1.003 (0.904-1.112)	.959
Prealbumin (mg/dL)	1.101 (1.011-1.199)	.027	1.032 (0.956-1.114)	.425	0.985 (0.974-0.996)	.007
FIB (g/L)	0.910 (0.717-1.156)	.441	0.893 (0.703-1.135)	.357	0.820 (0.641-1.049)	.114
IgG (g/L)	0.907 (0.841-0.978)	.011	0.937 (0.874-1.005)	.070	0.905 (0.839-0.977)	.010
IgA (g/L)	0.798 (0.987-1.095)	.798	0.913 (0.726-1.148)	.437	0.892 (0.706-1.128)	.339
IgM (g/L)	0.868 (0.603-1.249)	.445	0.939 (0.667-1.322)	.719	0.917 (0.645-1.304)	.631
C3 (g/L)	0.957 (0.828-1.106)	.549	0.656 (0.141-3.058)	.592	0.953 (0.800-1.135)	.591
C4 (g/L)	0.824 (0.459-1.477)	.515	0.809 (0.410-1.600)	.543	0.779 (0.299-2.030)	.610
RF (KU/L)	1.000 (0.999-1.000)	.582	1	.828	1.000 (0.999-1.000)	.711
CRP (mg/L)	1.003 (0.997-1.010)	.357	1.007 (0.999-1.015)	.069	0.997 (0.991-1.004)	.451
Hypertension	0.642 (0.307-1.342)	.239	0.977 (0.470-2.029)	.950	0.613 (0.293-1.282)	.193
Lung disease	1.330 (0.602-2.939)	.480	1.130 (0.513-2.491)	.761	1.277 (0.578-2.820)	.546
Diabetes	0.556 (0.224-1.382)	.207	1.038 (0.428-2.519)	.933	0.430 (0.169-1.090)	.075
Infection	0.665 (0.288-1.534)	.339	1.137 (0.498-2.598)	.761	0.532 (0.228-1.239)	.144
NEU ($10^9/\text{L}$)	0.998 (0.872-1.143)	.981	1.010 (0.881-1.156)	.890	1.029 (0.898-1.179)	.679
LYM ($10^9/\text{L}$)	0.964 (0.626-1.485)	.868	0.692 (0.434-1.104)	.122	1.257 (0.805-1.961)	.314
ESR (mm/H)	0.992 (0.981-1.002)	.992	0.990 (0.979-1.001)	.068	0.532 (0.228-1.239)	.144

indirect bilirubin has significantly difference between DAS28 $\leq$ 3.2 and DAS28>3.2. It is consistent with previous findings that ROS, partially regulated by bilirubin, was positively associated with disease activity. The exact protective effect of bilirubin might be contributed to its potent antioxidative property for inhibiting oxidation damage from ROS that hampered the production of autoantibodies, and powerful

immunomodulatory activity for inducing apoptosis of immune cells, down-regulating the expression of MHC-II molecules, and promoting Treg expansion.^{17,18} The HLA-DR4 has been discovered as the susceptibility factor for RA in large number of studies.¹⁰ Theoretical docking studies by Hideto Isogai revealed that bilirubin has the affinity to HLA-DR4 molecular, resulting in blocking the binding of

TABLE 4 Multiple logistic analysis the association between serum bilirubin levels and clinical and laboratory characteristics in rheumatoid arthritis patients

	Total bilirubin		Direct bilirubin		Indirect bilirubin	
	OR	P	OR	P	OR	P
Albumin (g/L)	—	—	—	—	0.991 (0.753-1.303)	.947
TBA (μmol/L)	—	—	—	—	1.443 (0.926-2.249)	.105
A/G	0.140 (0.002-8.057)	.342	—	—	0.150 (0.000-175.287)	.598
CHE (KU/L)	—	—	—	—	0.957 (0.547-1.674)	.877
Prealbumin (mg/dL)	1.166 (1.013-1.343)	.033	—	—	1.212 (1.034-1.421)	.018
IgG (g/L)	0.875 (0.743-1.031)	.111	0.944 (0.878-1.014)	.114	0.908 (0.711-1.160)	.442
CRP (mg/L)	—	—	1.010 (1.000-1.019)	.049	—	—
ESR (mm/H)	—	—	0.991 (0.978-1.005)	.206	—	—

TABLE 5 Correlation between serum bilirubins and laboratory data

	Total bilirubin		Direct bilirubin		Indirect bilirubin	
	r	P	r	P	r	P
Charlson	.005	.951	-.061	.488	.044	.621
Age (y)	-.106	.232	-.148	.093	-.058	.514
Total protein (g/L)	-.091	.307	-.129	.145	-.051	.566
Albumin (g/L)	.192	.029	.038	.671	.254	.004
A/G	.293	.001	.147	.097	.329	.000
Prealbumin (mg/dL)	.357	.004	.114	.379	.433	.000
TBA (μmol/L)	.172	.052	.129	.146	.188	.033
CHE (KU/L)	.162	.087	-.036	.707	.239	.011
Ada (U/L)	-.010	.926	.115	.293	-.081	.458
LP(a; mg/L)	-.011	.920	-.130	.240	.060	.586
APOA (g/L)	.273	.012	.086	.437	.334	.002
APOB (g/L)	.233	.033	.065	.559	.260	.017
LDL-c (mmol/L)	.136	.148	-.027	.772	.181	.054
HDL-c (mmol/L)	.186	.046	.017	.858	.255	.006
TCHO (mmol/L)	.178	.057	-.050	.598	.256	.006
TG (mmol/L)	.080	.393	-.059	.531	.136	.146
FIB (g/L)	-.149	.134	-.134	.180	-.166	.095
IgG (g/L)	-.338	.001	-.230	.027	-.337	.001
IgA (g/L)	-.023	.827	-.002	.986	-.072	.500
IgM (g/L)	.015	.892	.034	.755	.022	.84
C3 (g/L)	.081	.443	-.067	.531	.141	.184
C4 (g/L)	.150	.158	.116	.279	.117	.273
RF (KU/L)	-.069	.462	-.066	.487	-.044	.645
CRP (mg/L)	-.037	.689	.116	.214	-.134	.149
Hypertension	-.107	.228	-.003	.976	-.112	.208
Lung disease	-.003	.975	-.027	.762	.016	.853
Diabetes	-.103	.246	-.020	.821	-.120	.175
Infection	-.071	.419	.005	.951	-.069	.440
NEU (10 ⁹ /L)	.044	.621	-.030	.735	.062	.491
LYM (10 ⁹ /L)	-.012	.889	-.146	.103	.027	.763
ESR (mm/H)	-.266	.010	-.141	.113	-.223	.008

antigenic peptides to the HLA molecular to inhibit the immune response.¹⁰ Increasing the level of bilirubins in RA patients might be an effective method to control joint inflammation. However, although the therapy effects of bilirubin have been confirmed in a collagen-induced arthritis models, the toxicity of high concentrations of bilirubin should be taken into consideration in clinical use.

As we know, indirect bilirubin is not water soluble and bound to albumin in the blood. Consistent with our study, albumin was significantly decreased in RA patients and exhibited a positive association with serum indirect bilirubin. Albumin, another serum antioxidant, has been confirmed inversely associated with activation of proinflammatory cytokines.^{19,20} Hence, we proposed that both lower levels of bilirubin and albumin exerted an additive effect on the disease progression of RA and exacerbated the chronic inflammation. We also detected inverse association between total, indirect bilirubin and ESR, but not any correlation with other inflammatory markers, such as CRP, LYM, and NEU. Interestingly, our present study showed a significantly negative association of serum bilirubin with IgG. This phenomenon might be due to the inhibition of the complement cascade by blocking binding of C1 complex to antibody and regulating the expression of Fc receptors on macrophages.²¹ But strangely, the correlations between RF, C3, C4, IgA, IgM, and bilirubin have been not detected. The reason remained unknown and might be due to the small samples. Moreover, HDL-C, which binding with the S1P protein to suppress the lymphocyte cells proliferation and inflammatory responses, is significantly correlated with bilirubin.

However, the retrospective cross-sectional design and a single-center data in this study should be considered as limitations. Because of lack of follow-up, we could not evaluate the prognostic role of serum bilirubin on RA. To avoid the potential interference of confounding factors, only RA patients without any complications were included. Then, the sample size was relatively small. Therefore, a large sample in the future should be needed to certify the significance in RA patients.

In view of our observation and those from other findings, it is supported that serum bilirubin has a protective effect on RA, since the levels of bilirubin were significantly decreased in RA patients. This suggests that strategies to inducing biosynthesis of bilirubin might be partially preventing the RA progression. However, safe and persistent interventions to induce bilirubin should be taken into consideration.

AUTHOR CONTRIBUTIONS

All the authors have accepted responsibility for the entire content of this submitted article and approved submission.

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