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ORIGINAL ARTICLE

Decreased Bilirubin is Associated With Disease Activity of Primary Sjögren's Syndrome

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ABSTRACT

Objectives: This study aims to determine the serum bilirubin levels in primary Sjögren's syndrome (pSS) patients and to explore clinical significance of bilirubin in pSS.

Patients and methods: Retrospective analysis of electronic medical records was performed in 97 pSS patients (12 males, 85 females; mean age 54±15 years; range, 15 to 91 years) and 100 healthy controls (17 males, 83 females; mean age 51±14 years; range, 25 to 75 years). Serum bilirubin and other variables were compared between pSS patients and healthy controls. The European League Against Rheumatism Sjögren's syndrome disease activity index (ESSDAI) was used to assess the disease activity of pSS, and ESSDAI ≥5 was defined as moderate to high activity. The relationship between bilirubin and ESSDAI was analyzed by Spearman's correlation analysis and multivariable logistic regression.

Results: The median level of serum bilirubin was 9 μmol/L (interquartile range (IQR), 7-13 μmol/L) in pSS patients, much lower than healthy controls (median, IQR, 13, 10-18 μmol/L) ($p<0.001$). It was positively correlated with age ($r=0.255$, $p=0.012$), but negatively with immunoglobulin (Ig) A ($r=-0.314$, $p=0.003$), IgG ($r=-0.265$, $p=0.015$), erythrocyte sedimentation rate ($r=-0.309$, $p=0.002$) and ESSDAI ($r=-0.342$, $p=0.001$). Multivariate analysis revealed that increased bilirubin was independently associated with decreased risk of moderate to high disease activity (odds ratio, 95% confidence interval: 0.852, 0.730-0.955).

Conclusion: Serum bilirubin is decreased in pSS patients and may be a useful biomarker for reflecting pSS disease activity.

Keywords: Bilirubin, disease activity, primary Sjögren's syndrome.

Primary Sjögren's syndrome (pSS) is a common systemic autoimmune disease of unknown cause, which predominantly affects females.[1] Although pSS is characterized by autoimmune inflammation of exocrine glands leading to dryness of eyes and mouth, systemic manifestations vary between pSS patients and this heterogeneity is difficult to reflect in the underlying pathogenesis. To date, two indices for disease activity assessment in pSS have been validated by The European League Against Rheumatism (EULAR) task force, namely the EULAR Sjögren's syndrome disease activity index (ESSDAI) and EULAR Sjögren's syndrome patient-reported index (ESSPRI).[2-5] The former is used for assessing systemic features and the latter for patients' symptoms. The sensitivity to disease change is better for ESSDAI than ESSPRI. However, both of the two indices have some limitations, including the subjectivity dependent on physician assessment or patients' response, and the difficulty of calculating ESSDAI or ESSPRI. Accordingly, it is necessary to search for more simple and objective biomarkers of pSS disease activity.

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Accumulating evidence suggested the occurrence of a prooxidant state in SS patients and involvement of oxidative stress in SS pathogenesis.[6-9] Bilirubin, a major breakdown product of heme catabolism, is a potent antioxidant with immunomodulatory and antiinflammatory activity at low physiological concentrations.[10,11] Moreover, bilirubin showed a stronger antioxidative function than many other antioxidants, including alpha-tocopherol, ascorbic acid, and catalase.[12,13] Recently, decreased levels of serum bilirubin have been linked to some connective tissue diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), polymyositis (PM), the pathogenesis of which involves oxidative stress and inflammatory injury.[14-19] However, to our knowledge, there has been no study investigating whether serum bilirubin is decreased in pSS patients. Therefore, in this study, we aimed to determine the serum bilirubin levels in pSS patients and to explore clinical significance of bilirubin in pSS.

PATIENTS AND METHODS

A total of 97 newly diagnosed patients with pSS (12 males, 85 females; mean age 54±15 years; range, 15 to 91 years) in Changzheng Hospital were consecutively recruited in this study between January 2006 and December 2014. The diagnosis of pSS was based on the American-European Consensus Group criteria for pSS.[20] Exclusion criteria were age <18 years, diagnosis of another autoimmune or inflammatory disease such as SLE, RA, PM/dermatomyositis, inflammatory bowel diseases, diabetes, cardiovascular diseases, etc., malignancies, liver diseases, hematology diseases or administration of blood transfusion in the past four months, or any pSS-related medical treatment. The healthy control group included 100 healthy individuals (17 males, 83 females; mean age 51±14 years; range, 25 to 75 years) undergoing routine physical examinations in the hospital during the same period. They had no such significant underlying diseases as autoimmune disease, liver or renal disease, malignancies, hematologic diseases or diabetes.

Demographic, clinical and laboratory data were extracted from electronic medical records. ESSDAI of each pSS patient was calculated in accordance with 12 domains (i.e. constitutional, lymphadenopathy, glandular, articular, cutaneous, pulmonary, renal, muscular, peripheral nervous system, central nervous system, hematological, biological domains).[2] Of note, blood samples were drawn and used for laboratory analysis after an overnight fasting of at least eight hours. The study protocol was approved by the Changzheng Hospital Ethics Committee. In accordance with the policy of our institution, written informed consents were not required, since this study using data from routine medical records had no influence on subsequent management of patients. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Statistical analysis was performed using the PASW version 17.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean±standard deviation for normal distribution and median and interquartile range (IQR) for non-normal distribution. Categorical variables were described as frequencies. Student t-test or Mann-Whitney U test was used to compare continuous variables if appropriate. Categorical variables were compared by Chi square (χ^2) test. Spearman's correlation analysis was used for assessing the correlation between bilirubin and other continuous variables. Multivariate logistic regression was performed with inclusion of variables with $p<0.05$ in the univariate analyses. Results were considered statistically significant when p value was <0.05.

RESULTS

The clinical and laboratory characteristics of participants were shown in Table 1. The age and sex composition were comparable between pSS patients and healthy controls ($p>0.05$). White blood cell (WBC) count was significantly decreased in pSS patients. The median level of serum bilirubin was 9 μmol/L (IQR, 7-13 μmol/L) in pSS patients, much lower than healthy controls (median, IQR, 13, 10-18 μmol/L) ($p<0.001$) (Figure 1).

Mann-Whitney U test was used to analyze the association between serum bilirubin and categorical variables. The results showed that the

Table 1. Clinical and laboratory characteristics of study subjects

	pSS patients (n=97)				Healthy controls (n=100)					
	n	Mean±SD	Median	Min-Max	n	Mean±SD	Median	Min-Max	Reference range	*p*
Age (year)		54±2								0.140
Sex						51±1				0.359
Male	12				17					
Female	85				83					
WBC count ($\times 10^9$/L)		5.3±2.5				6.1±1.2			4-10	0.003
IgA (g/L)			2.71	2.00-3.77					0.82-4.53	
IgM (g/L)			1.33	0.84-1.90					0.46-3.04	
IgG (g/L)			16.85	12.45-24.13					7.51-15.60	
C3 (g/L)			0.83	0.70-1.02					0.79-1.52	
C4 (g/L)			0.18	0.15-0.21					0.16-0.38	
RF (U/ml)			20	11-73					0-20	
ESR (mm/h)			38	16-74					1-20	
CRP (mg/L)			2.92	1.28-7.23					0-10	
ALT (U/L)			16	11-24			16	14-24	0-40	0.355
AST (U/L)			21	17-28			20	17-24	0-35	0.136
ANA										
+	75									
-	22									
Anti-SSA										
+	72									
-	25									
Anti-SSB										
+	45									
-	52									
ACA										
+	9									
-	88									
ESSDAI			5	3-8						

pSS: Primary Sjögren's syndrome; SD: Standard deviation; Min: Minimum; Max: Maximum; WBC: White blood cell; Ig: Immunoglobulin; C: Complement component; RF: Rheumatoid factor; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; ALT: Alanine aminotransaminase; AST: Aspartate aminotransferase; ANA: Antinuclear antibodies; Anti-SSA: Anti-Sjögren's syndrome type A antibody; Anti-SSB: Anti-Sjögren's syndrome type B antibody; ACA: Anti-centromere antibodies; ESSDAI: The European League Against Rheumatism Sjögren's syndrome disease activity index.

levels of serum bilirubin were significantly increased in pSS patients with anti-Sjögren's syndrome type B (anti-SSB) antibody compared with those without (p=0.016), while they were not associated with sex, antinuclear antibodies, anti-centromere antibodies, and anti-Sjögren's syndrome type A antibody (Table 2). Spearman's correlation analysis was used for analyzing the correlation between bilirubin and continuous variables. The results indicated that serum bilirubin was positively correlated with age (r=0.255, p=0.012), but negatively with immunoglobulin (Ig) A (r=-0.314, p=0.003), IgG (r=-0.265, p=0.015), erythrocyte sedimentation rate (ESR) (r=-0.309, p=0.002) and ESSDAI (r=-0.342, p=0.001) (Table 3). There

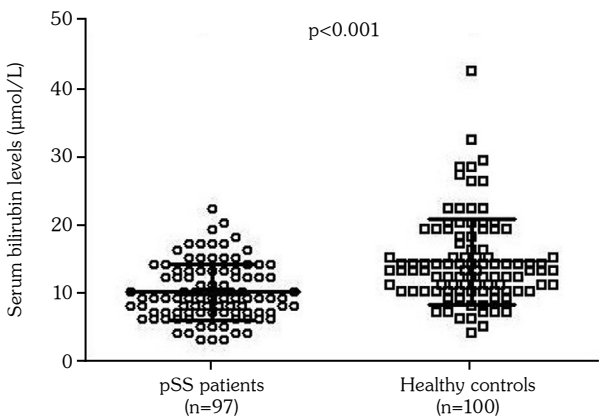

Figure 1. Serum bilirubin levels in pSS patients and healthy controls.
pSS: Primary Sjögren's syndrome.

Table 2. Relationship between serum bilirubin and categorical variables

	Bilirubin (μmol/L)		
	Median	Min-Max	*p*
Sex			0.895
Male	9.0	7.5-11.8	
Female	9.0	7.0-13.0	
ANA			0.122
Positive	9.0	7.0-12.0	
Negative	12.5	8.8-15.0	
Anti-SSA			0.137
Positive	9.0	7.0-12.0	
Negative	10.0	7.5-15.0	
Anti-SSB			0.016
Positive	8.0	6.0-10.5	
Negative	10.0	8.0-14.0	
ACA			0.485
Positive	10.0	8.5-13.0	
Negative	9.0	7.0-13.0	

ANA: Antinuclear antibodies; Anti-SSA: Anti-Sjögren's syndrome type A antibody; Anti-SSB: Anti-Sjögren's syndrome type B antibody; ACA: Anti-centromere antibodies.

Table 3. Correlation between bilirubin and other continuous variables in pSS patients

	Bilirubin	
	r	*p*
Age	0.255	0.012
White blood cell count	-0.121	0.239
Immunoglobulin A	-0.314	0.003
Immunoglobulin M	0.098	0.371
Immunoglobulin G	-0.265	0.015
Complement component 3	0.025	0.818
Complement component 4	0.081	0.458
Rheumatoid factor	0.007	0.957
Erythrocyte sedimentation rate	-0.309	0.002
C-reactive protein	-0.190	0.069
Alanine aminotransaminase	0.107	0.297
Aspartate aminotransferase	0.040	0.702
ESSDAI	-0.342	0.001

pSS: Primary Sjögren's syndrome; ESSDAI: The European League Against Rheumatism Sjögren's syndrome disease activity index.

was no significant correlation between serum bilirubin and WBC count, IgM, complement components 3 and 4, rheumatoid factor, C-reactive protein, alanine aminotransaminase and aspartate aminotransferase.

Since aforementioned univariate analysis showed that bilirubin was associated with anti-SSB antibody, age, IgA, IgG and ESR, we performed a multivariate logistic regression analysis including these indices to explore the independent association between bilirubin and disease activity. Inactivity or low activity was defined as ESSDAI <5 and moderate or high activity as ESSDAI ≥5. The results indicated that serum bilirubin was independently associated with disease activity (odds ratio, 95% confidence interval: 0.852, 0.730-0.955) after adjusting for confounders (Table 4). Each increase per μmol/L in serum bilirubin was associated with a significant 14.8% reduction in risk of moderate to high activity.

DISCUSSION

This is, to the best of our knowledge, the first study to investigate the level of serum bilirubin in pSS patients and the association of decreased bilirubin with pSS disease activity. In the present study, we found that the level of serum bilirubin was

Table 4. Multivariate logistic analysis of association with disease activity

	ESSDAI ≥5		
	OR	95% CI	*p*
Age	1.005	0.959-1.054	0.822
Immunoglobulin A	0.908	0.702-1.173	0.459
Immunoglobulin G	1.057	0.945-1.181	0.331
Erythrocyte sedimentation rate	1.035	1.006-1.064	0.016
Bilirubin	0.852	0.730-0.955	0.043
Anti-Sjögren's syndrome type B antibody	0.211	0.118-1.604	0.435

ESSDAI: The European League Against Rheumatism Sjögren's syndrome disease activity index; OR: Odds ratio; CI: Confidence interval.

decreased in pSS patients and that the decrease of bilirubin was closely associated with higher pSS disease activity, as measured by ESSDAI. Our findings indicated that serum bilirubin may be a potential index to estimate pSS disease activity.

The antioxidant property of bilirubin has been well recognized.[10-13] Besides, bilirubin of physiological concentration also has immunomodulatory effects on antigen-presenting cells, effector cells and T helper 17 (Th17) cells, ultimately favoring the expansion of forkhead box protein 3+ (FOXP3+) Tregs.[21-25] A number of studies found that bilirubin is a protective factor and decreased in several inflammatory or autoimmune diseases such as atherosclerosis, SLE, RA, PM, and diabetic nephropathy.[14-19,26,27] pSS is also a systemic autoimmune disease. It has been suggested that various immune effectors (such as Th1, Th17, T follicular helper, and B cells) may be involved in the pathogenesis of different phases in pSS.[28] In addition, it has been proved that there is a prooxidant state in pSS patients and that oxidative stress may be an important factor in the pathogenesis of pSS.[6-9] Combined with the aforementioned biological activity of bilirubin and pathogenesis of pSS, our present findings of decreased bilirubin in pSS patients suggested that bilirubin may also play a protective role in pSS by antioxidation and immunoregulation, although the causality between the decrease of bilirubin and the development of pSS remains to be elucidated.

Another finding of this study is that serum bilirubin was inversely associated with disease activity and performed well in discriminating between low and moderate to high activity. This finding suggested that serum bilirubin may be a useful index for estimating pSS disease activity. Compared with ESSDAI and ESSPRI that were the only two indices validated by EULAR, serum bilirubin may be more simple and objective, and less likely to be affected by the experience of clinicians. Since the measurement of serum bilirubin is easy and inexpensive, it may be of clinical significance to measure serum bilirubin level in each pSS patient in routine tests. Furthermore, appropriate use of bilirubin may be a simple and cost-effective method for pSS treatment. Admittedly, how to use the bilirubin needs to be practiced in future studies.

Some limitations of this study should be addressed. Firstly, this is a cross-sectional study, in which the causal relationship between low bilirubin and pSS cannot be confirmed. Further prospective studies are needed to confirm the causality. Secondly, since this is a single-center study with small size, the results that may be biased should be interpreted in caution. Thirdly, due to the lack of more detailed clinical data, some factors such as smoking, drinking, and body mass index were not included in our multivariate analysis, which, possibly more or less, have some effects on our results.

In conclusion, the current study demonstrated that serum bilirubin was decreased in pSS patients and this decrease was independently associated with pSS disease activity. Bilirubin may be a simple, objective and useful biomarker for reflecting pSS disease activity.

Declaration of conflicting interests

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