Serum Bilirubin Concentrations in Patients With Takayasu Arteritis

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Context.—Bilirubin has strong anti-inflammatory and antioxidative stress action. Progression of inflammation involving arteries is a crucial activator in pathogenesis of Takayasu arteritis (TA).

Objective.—To investigate the relationship between serum bilirubin and TA.

Design.—Our study involved 115 consecutive TA patients. Patients with active-phase disease were followed and received prednisone therapy.

Results.—Lower concentrations of serum bilirubin were detected in TA patients compared with healthy subjects (0.6 ± 0.31 versus 0.7 ± 0.22 mg/dL, P = .02). Serum bilirubin concentrations in active TA patients were lower than those in inactive patients (0.5 ± 0.20 versus 0.8 ± 0.32 mg/dL, P < .001). In all patients with TA, serum bilirubin correlated positively with total protein (r = 0.193, P = .04) and negatively with C-reactive protein and erythrocyte sedimentation rate (r = −0.213, P = .03, and r = −0.532, P < .001, respectively). Multiple logistic regression analysis showed that each decrease of 1 mg/dL in serum bilirubin was associated with a 1.10 times increase in the odds for TA compared with the controls (odds ratio = 0.913, 95% CI, 0.856–0.974; P = .006). Serum bilirubin was correlated with erythrocyte sedimentation rate (β = −0.170, P < .001) in multiple linear regression analysis. The area under the curve for serum bilirubin in predicting active TA patients was 0.802. Serum bilirubin levels were found to be significantly increased after prednisone treatment (0.5 ± 0.20 versus 0.7 ± 0.15 mg/dL, P = .002).

Conclusions.—Lower serum bilirubin levels are associated with TA, and serum bilirubin may be influenced by prednisone therapy in active TA patients. Serum bilirubin levels in TA patients correlate negatively with erythrocyte sedimentation rate.

(Takayasu arteritis (TA) is a chronic, recurrent, inflammatory vasculitis characterized by granulomatous inflammation in the vessel wall, and mainly affects young females. The aortic arch and its primary branches, such as the ascending aorta, abdominal aorta, and thoracic descending aorta, are primarily implicated in patients with TA. In the clinical laboratory, some inflammatory markers such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and tumor necrosis factor (TNF) have been associated with disease progression. There is growing evidence that progression of inflammation involving arteries is a crucial activator in the pathogenesis of TA, which results in occlusion, dilatation, and segmental stenosis. Obviously, it is important to estimate inflammatory conditions for the management of patients with TA.

Antioxidative enzymes and antioxidative substances play important roles in the oxidative stress and inflammation defense system. Bilirubin, as a product generated by heme metabolism, is an effective scavenger of free radicals and strong endogenous antioxidants factors in the body. In some oxidative stress-mediated diseases, serum bilirubin concentrations are inversely correlated with cardiovascular diseases. Very recently, lower serum bilirubin concentrations have been also reported in patients with carbon monoxide poisoning, migraine, and pulmonary embolism. Notably, several recent studies found bilirubin to be significantly related to several rheumatic diseases, including systemic lupus erythematosus, polymyositis, and rheumatoid arthritis. These studies provide evidence that oxidative stress and inflammation may tend to alter serum bilirubin concentrations. It is known that inflammatory cells can infiltrate and localize in the adventitia in patients with TA, and few reliable clinical markers are available to reflect the progression and remission of TA patients. Therefore, the goal of this study was to investigate the relationship between serum bilirubin and TA.

MATERIALS AND METHODS

Patient Selection

Our study involved 115 consecutive patients with TA from Affiliated Hospital of Youjiang Medical University for Nationalities (Baise, China) between January 2014 and July 2016. These patients with TA were diagnosed in accordance with the American College of Rheumatology classification criteria. The present study excluded patients who had cardiovascular disease, hepatic or renal insufficiency, dyslipidemia, infectious disease, metabolic disease, malignancy, smoking, or other rheumatic diseases. The control groups consisted of 193 sex- and age-matched healthy subjects.

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The study's protocol was approval by the Ethics Committee of Affiliated Hospital of Youjiang Medical University for Nationalities; all subjects signed informed consent forms.

**Laboratory and Clinical Investigation**

Laboratory parameters and clinical data were obtained from the medical records. Body mass index was calculated by dividing weight in kilograms by height in meters squared. Fasting blood samples were collected for these laboratory measurements. Values of serum alanine aminotransferase, aspartate aminotransferase, creatinine, total protein (TP), glucose, and bilirubin concentrations were measured on an identical automatic analyzer with standard assays. Serum total bilirubin was measured by using the diazo method (Roche Inc, Basel, Switzerland). The serum concentrations of CRP were determined with immunonephelometry, and ESR was measured using the traditional Westergren method. Clinical characteristics such as disease duration, hypertension, diabetes mellitus, and medication history were summarized in all patients.

**Disease Activity Assessment**

According to the National Institutes of Health criteria, active patients with TA were defined by the following features: (1) typical clinical symptoms, (2) vascular insufficiency signs, (3) typical angiographic characteristics, and (4) increased ESR with no infection. According to the criteria, there were 54 patients in the active phase of the disease and 61 patients in the inactive phase at the outset of this study.

**Follow-up of Active Patients**

The patients who were diagnosed with the active phase of the disease were followed and received prednisone therapy (1 mg/kg per day). Concomitant medications were not used during the period of the study. Responders were defined by the following clinical remission criterion: (1) disappearance or decrease of clinical symptoms, (2) no significant disease progress in vascular imaging findings, and (3) ESR within normal values. Further, laboratory and clinical data were compared in active TA patients before and after treatment.

**Statistical Analysis**

Statistical analysis was performed with SPSS 16.0 statistical software (SPSS Inc, Chicago, Illinois). The Kolmogorov-Smirnov test was used to determine normal distribution. The Student t test or Mann-Whitney U test for continuous variables and χ² test for categorical variables were used, as appropriate. We used the Spearman approach to evaluate the correlations between serum bilirubin and demographics or laboratory parameters. Multiple logistic regression analysis was used to calculate the odds of serum bilirubin concentrations in patients with TA compared with controls, and multiple linear regression analysis was used with statistical adjustments for underlying confounders. The receiver operating characteristic curve was used to estimate the performance of serum bilirubin in identifying active patients in all patients with TA. Finally, laboratory measurements before and after prednisone treatment were compared with paired t test. Statistical significance was set at P < .05.

**RESULTS**

The demographic and laboratory data for TA patients and healthy individuals are shown in Table 1. Most of these patients were found to have a medication history (prednisone) and hypertension (77.4% and 55.7%, respectively). The mean disease duration was 4.1 ± 6.72 years. The mean values for serum bilirubin, CRP, and ESR were 0.6 ± 0.31 mg/dL, 11.1 ± 5.32 mg/L, and 22.8 ± 15.13 mm/h, respectively, in all patients with TA (to convert serum bilirubin concentration to millimoles per liter, multiply by 88.4. To convert blood glucose concentration to millimoles per liter, multiply by 0.0555. To convert serum bilirubin concentration to millimoles per liter, multiply by 17.104).

All patients with TA were divided into active and inactive patients; clinical features and laboratory findings of these patients are reported in Table 2. At baseline, age, sex, body mass index, disease duration, medication history, diabetes mellitus, hypertension, alanine aminotransferase, aspartate aminotransferase, creatinine, TP, and fasting blood glucose did not differ between the 2 groups. Values of ESR and CRP in active patients with TA were significantly high as compared with those in inactive TA patients. Serum bilirubin concentrations in active TA patients were lower than those in inactive patients (0.5 ± 0.20 versus 0.8 ± 0.32 mg/dL, P < .001).

In all patients with TA, correlation analysis revealed that serum bilirubin correlated positively with TP (r = 0.193, P = .04), and negatively with CRP and ESR (r = -0.213, P = .03, and r = -0.532, P < .001, respectively). The correlation analysis was performed separately for active patients and inactive patients; the serum concentrations of bilirubin correlated negatively with ESR (r = -0.326, P = .02), and positively with TP (r = 0.348, P = .01) in active patients.
whereas no correlations with ESR or CRP were found in inactive patients.

Multiple logistic regression analysis showed that each decrease of 1 mg/dL in serum bilirubin was found to be associated with a 1.10 times increase in the odds for TA compared with the controls (odds ratio = 0.913; 95% CI, 0.856–0.974; P = .006). Multiple linear regression analysis was used with serum bilirubin as an objective variable and age, sex, disease duration, medication history, diabetes mellitus, hypertension, alanine aminotransferase, aspartate aminotransferase, TP, CRP, and ESR as explanatory variables, indicating that serum bilirubin was correlated with ESR (β = −0.170, P < .001) independently of other parameters in multiple linear regression analysis (Table 3). The area under the curve for serum bilirubin in estimating active TA patients was 0.802 (95% CI, 0.723–0.88; P = .002), whereas ESR and CRP were associated with TA independently of CRP and ESR, and active patients with TA showed significantly lower serum bilirubin levels compared with patients with inactive disease. Moreover, serum bilirubin correlated negatively with ESR in all patients with TA in multiple linear regression analysis. Surprisingly, serum bilirubin levels of active patients were found to be increased after prednisone therapy.

Lower serum bilirubin concentrations have been associated with coronary atherosclerosis and cardiovascular disease. It has also been shown that serum concentrations of bilirubin are related to chronic kidney disease, cardioemboic stroke, and severe sepsis. Our study revealed the association between lower serum levels of bilirubin and TA. Bilirubin as a novel biochemical tool has been regarded as a potent antioxidant, and bilirubin has much stronger anti-inflammatory and antioxidative stress action than many other antioxidants. A reverse relationship between low serum bilirubin levels and CRP has

### DISCUSSION

Clinically, imaging examinations are important and indispensable for the diagnosis of TA, and the presence of clinical symptoms and signs such as ischemia, vascular murmur, hypertension, disappeared or weakened pulsation, and large-vessel inflammation contributes to help identify patients with TA. In addition, CRP and ESR have distinct and complementary roles for establishing the diagnosis of TA. In our study, the serum concentrations of bilirubin were associated with TA independently of CRP and ESR, and active patients with TA showed significantly lower serum bilirubin levels compared with patients with inactive disease. Moreover, serum bilirubin correlated negatively with ESR in all patients with TA in multiple linear regression analysis. Surprisingly, serum bilirubin levels of active patients were found to be increased after prednisone therapy.

### Table 2. Clinical and Laboratory Features in Active and Inactive Patients

|                        | Active Patients | Inactive Patients | P   |
|------------------------|-----------------|-------------------|-----|
| Female, No. (%)        | 53 (98.1)       | 57 (93.4)         | .44 |
| Age, y                 | 27.2 ± 8.42     | 28.5 ± 8.82       | .41 |
| Body mass index, kg/m² | 25.5 ± 3.24     | 24.7 ± 3.24       | .18 |
| Disease duration, y    | 4.5 ± 7.64      | 3.7 ± 4.78        | .51 |
| Medication history     | 44 (81.5)       | 45 (73.8)         | .32 |
| Diabetes mellitus, No. (%) | 0 (0)        | 2 (3.3)           | .53 |
| Hypertension, No. (%)  | 32 (59.3)       | 32 (52.5)         | .46 |
| C-reactive protein, mg/L | 18.4 ± 21.28   | 4.7 ± 3.93        | <.001|
| Erythrocyte sedimentation rate, mm/h | 3.7 ± 6.47 | 12.1 ± 5.22 | <.001|
| Aspartate aminotransferase, U/L | 21.9 ± 12.36 | 18.4 ± 8.43 | .08 |
| Creatinine, mg/dL      | 20.0 ± 9.89     | 17.5 ± 5.66       | .11 |
| Total protein, g/dL    | 0.7 ± 0.17      | 0.7 ± 0.15        | .21 |
| Fasting blood glucose, mg/dL | 86.3 ± 29.22  | 80.6 ± 9.46       | .17 |
| Serum bilirubin, mg/dL | 0.5 ± 0.20      | 0.8 ± 0.32        | <.001|

SI conversion factors: To convert creatinine concentration to millimoles per liter, multiply by 88.4. To convert blood glucose concentration to millimoles per liter, multiply by 0.0555. To convert serum bilirubin concentration to millimoles per liter, multiply by 17.104.

### Table 3. Impact of Multiple Factors on Serum Bilirubin Concentrations in Patients With Takayasu Arteritis

|                        | Unstandardized Coefficients |            |            |            |            |
|------------------------|-----------------------------|------------|------------|------------|------------|
|                        | β (t,P)                     | SE (β)     |           |            |            |
| Sex                    | −2.498 (t = −0.066, P = .49) | 3.612      | −0.692     | .43        |
| Age                    | −0.015 (t = −0.023, P = .89) | 0.109      | −0.134     | .20        |
| Disease duration       | 0.113 (t = 0.131, P = .42)  | 0.139      | 0.809      | .19        |
| Hypertension           | 0.813 (t = 0.077, P = .43)  | 1.022      | 0.796      | .37        |
| Diabetes mellitus      | 4.613 (t = 0.087, P = .37)  | 5.105      | 0.904      | .76        |
| Medication history     | −1.231 (t = −0.097, P = .30) | 1.177      | −1.045     | .00        |
| C-reactive protein     | −0.070 (t = −0.210, P = .12) | 0.045      | −0.157     | <.001      |
| Erythrocyte sedimentation rate | −0.170 (t = −0.506, P = .001) | 0.046      | −3.732     | <.001      |
| Aspartate aminotransferase | 0.109 (t = 0.212, P = .13)  | 0.072      | 1.518      | .19        |
| Aspartate aminotransferase | −0.132 (t = −0.208, P = .14) | 0.088      | −1.505     | .14        |
| Total protein          | 0.161 (t = 0.251, P = .03)  | 0.071      | 2.264      | .07        |
been reported in apparently healthy adults, indicating that bilirubin metabolism has antioxidant and anti-inflammatory effects. Current evidence has also demonstrated that serum bilirubin can provide important protection against inflammation. Furthermore, serum bilirubin can suppress TNF-α–related induction in endothelial adhesion molecules, and presents a protective action against inflammatory progression. Accumulating data have indicated that serum bilirubin positively correlates with the total antioxidant capacity in the human body. In view of the above summary, the protective role of bilirubin against inflammation may be a potential mechanism to explain lower serum bilirubin levels in active patients with TA, because bilirubin may be destroyed by strong inflammation and oxidative stress, and excessive inflammatory response may result in primary consumption and deficit of bilirubin, which may be associated with lower serum bilirubin concentrations in TA patients in the active phase.

Compared with imaging examinations, serum bilirubin as a component of biochemical tests is an available and simple marker with no additional costs for patients in clinical practice, and serum bilirubin is more convenient for the assessment of inflammatory progression compared with angiography in patients with TA. An increase in serum bilirubin levels was observed after successful prednisone therapy in active patients with TA, suggesting that treatment with anti-inflammatory medication in active patients can influence bilirubin metabolism because of alleviated inflammation. Accordingly, the results indicated that serum bilirubin may be considered as a biomarker to estimate prednisone treatment outcome in TA patients in the active phase.

The main limitation of our study is a small sample for the relatively rare disease, especially for the active patients with prednisone therapy during the follow-up period. Second, several genes with gene coding for uridine diphosphate–glucuronosyltransferase are involved in the regulation of serum bilirubin levels by the biliary catabolic pathway. However, genomic DNA from patients with TA was not available in the current study. Third, a single measurement for all patients with TA did not estimate intra-individual variability. Finally, the correlation between serum bilirubin concentrations and disease classification of patients with TA was not analyzed. In summary, our study suggests lower serum bilirubin levels are associated with TA, and serum bilirubin may be influenced by prednisone therapy in active TA patients. Serum bilirubin levels in TA patients correlate negatively with ESR. Nevertheless, our results need confirmation with larger samples.

Table 4. Comparison of Laboratory Features in Active Patients Before and After Prednisone Therapy

|                     | Pretreatment       | Posttreatment      | P     |
|---------------------|--------------------|--------------------|-------|
| C-reactive protein, mg/L | 17.1 ± 21.18        | 5.0 ± 4.07         | .007  |
| Erythrocyte sedimentation rate, mm/h | 34.3 ± 13.67        | 12.3 ± 5.41        | .001  |
| Alanine aminotransferase, U/L | 21.5 ± 12.38        | 16.9 ± 7.80        | .07   |
| Aspartate aminotransferase, U/L | 18.6 ± 7.08         | 16.5 ± 4.81        | .15   |
| Creatinine, mg/dL    | 0.7 ± 0.17          | 0.7 ± 0.15         | .05   |
| Total protein, g/dL  | 6.8 ± 0.95          | 6.8 ± 0.55         | .76   |
| Fasting blood glucose, mg/dL | 87.5 ± 25.95       | 80.7 ± 10.65       | .13   |
| Serum bilirubin, mg/dL | 0.5 ± 0.20          | 0.7 ± 0.15         | .002  |

Si conversion factors: To convert creatinine to concentration to millimoles per liter, multiply by 0.0555. To convert glucose concentration to millimoles per liter, multiply by 0.0595. To convert serum bilirubin concentration to millimoles per liter, multiply by 17.104.

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