A potential clinical usefulness of measuring serum bilirubin levels in patients with polymyositis

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Abstract. – OBJECTIVE: Accumulated data have indicated that bilirubin has antiinflammatory, antioxidative, and immunosuppressive properties. Polymyositis (PM) belongs to an autoimmune disease characterized by chronic inflammation in skeletal muscle. Until now, to the best of our knowledge, there are no literature investigating bilirubin levels in patients with PM. Therefore, the aim of this investigation was to assess a relationship between bilirubin and PM.

PATIENTS AND METHODS: Our study included newly diagnosed 77 patients with PM who were admitted to the Affiliated Hospital of Youjiang Medical University for Nationalities (Guangxi, China) and 108 healthy subjects as controls. Clinical characteristics and laboratory parameters of patients were analyzed, retrospectively.

RESULTS: The serum concentrations of total bilirubin (TB), conjugate bilirubin (CB), unconjugated bilirubin (UCB) were significantly lower in patients with PM than healthy controls. Serum concentrations of TB were negatively correlated with erythrocyte sedimentation rate (ESR), creatine kinase (CK) and lactic dehydrogenase (LDH) in patients with PM (r=-0.494, p<0.001; r=-0.274, p=0.017; r=-0.292, p=0.014), and serum concentrations of UCB were negatively correlated with ESR and CK in PM patients (r=-0.424, p<0.001; r=-0.234, p=0.041). Both serum TB and UCB concentrations were positively correlated with manual muscle test (MMT) score in patients with PM (r=0.328, p=0.004; r=0.333, p=0.004). In multiple linear regression analysis, serum UCB levels were independently associated with MMT score (r=0.239, p=0.003).

CONCLUSIONS: We observed significantly lower serum concentrations of TB, CB and UCB in patients with PM, and suggested a potential association between serum concentrations of UBC and disease activity in PM patients.

Key words: Serum bilirubin, Polymyositis, Disease activity.

Introduction

Polymyositis (PM) is an idiopathic inflammatory myositis with muscle weakness, and belongs to autoimmune disease characterized by chronic inflammation in skeletal muscle.1 Knowing that inflammation causes muscular weakness in patients with PM,2 and erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and tumor necrosis factor (TNF) were increased in patients with PM3-5. Some inflammatory cytokines such as interleukin-15, interleukin-17 and monocyte chemoattractant proteins have been found to be correlated with PM6. Importantly, Hak et al7 support a notion that immune-mediated inflammation is involved in the development of PM.

Serum bilirubin, as the intravascular product of heme catabolism, is a potent scavenger of reactive oxygen species.8 Recently, there is evidence suggests that serum bilirubin is considered to be a potent antioxidant providing important protection against inflammation in various diseases9,10. The protective properties have been demonstrated in patients with systemic lupus erythematosus, metabolic syndrome X and myasthenia gravis11-14. Numerous studies have demonstrated in the literature that serum concentrations of bilirubin have been reported to be correlated with diabetes mellitus, metabolic syndrome and Gilbert syndrome15,16. It has been well documented that lower serum concentrations of bilirubin are associated with multiple sclerosis, with estimated glomerular filtration rate in patients with diabetes, and with the adverse outcomes in patients with severe sepsis17-19. Despite these literatures have indicated the benefit of bilirubin in varied inflammation-related diseases, however, until now, to the best of our knowledge, there are no literature investiga-
ting bilirubin levels in patients with PM. Therefore, the aim of this investigation was to assess the relationship between bilirubin and PM.

**Patients and Methods**

Our study included newly diagnosed 77 patients with PM who were admitted to the Affiliated Hospital of Youjiang Medical University for Nationalities (Guangxi, China). Clinical characteristics and laboratory parameters of patients were collected in the archives of the hospital. The definition of PM was according to the Bohan and Peter criteria. The manual muscle test (MMT) was used to assess disease activity in patients with PM, which indicates full muscle strength. Age- and sex-matched 108 healthy subjects who underwent routine physical examinations in our hospital were considered as controls. The following patients were excluded from analyses: Known liver disease or liver injury, renal insufficiency, biliary disease (such as gallstones, cholecystitis and gallbladder polyps), diabetes, hypertension, smoking, cardiovascular disease, acute or chronic infection, hematological disorder, cancer and mental illness. In addition, patients with cancer-associated myositis and other systemic-associated autoimmune disease were also excluded to this study.

This study was conducted in accordance with the Declaration of Helsinki and approved by the Affiliated Hospital of Youjiang Medical University Institutional Review Board.

Serum concentrations of total bilirubin (TB), conjugate bilirubin (CB), unconjugated bilirubin (UCB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), creatine kinase (CK), lactic dehydrogenase (LDH) were measured by using automatic biochemical analysis (Inc., Roche, Switzerland), values of ESR were measured using natural sedimentation method, and the measure of serum CRP levels were performed by IMMAGE immunochemistry system (Inc., Beckman, Germany).

**Statistical Analysis**

Continuous variables were shown as mean ± standard deviation, and categorical variables were presented as percentages. We used Kolmogorov-Smirnov test to identify data normality. Continuous variables with normal distribution were analyzed by independent Student’s t-test, otherwise, non-normal distribution data were compared using Mann-Whitney U test. Chi-square test was used to analyze the differences in proportions between groups. The correlation between two continuous variables was analyzed using the Spearman approach. We used multiple linear regression analysis to assess the potential correlation between serum concentrations of TB, CB, UCB and disease activity in PM patients. Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA), and p<0.05 was determined as statistically significant.

**Table I.** Demographic characteristics and laboratory parameters of PM patients and healthy individuals.

<table>
<thead>
<tr>
<th></th>
<th><strong>PM patients</strong></th>
<th><strong>Controls</strong></th>
<th><strong>p-value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender (Male/Female) [n]</strong></td>
<td>22/55</td>
<td>34/74</td>
<td>0.671</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>47.1±14.08</td>
<td>44.9±6.37</td>
<td>0.207</td>
</tr>
<tr>
<td><strong>CRP (mg/L)</strong></td>
<td>8.3±16.94</td>
<td>——</td>
<td>——</td>
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<tr>
<td><strong>ESR (mm/h)</strong></td>
<td>20.3±17.57</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td><strong>ALP (U/L)</strong></td>
<td>72.6±29.39</td>
<td>68.7±23.78</td>
<td>0.320</td>
</tr>
<tr>
<td><strong>ALT (U/L)</strong></td>
<td>72.6±31.49</td>
<td>27.6±18.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>AST (U/L)</strong></td>
<td>40.9±31.50</td>
<td>21.5±11.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>GGT (U/L)</strong></td>
<td>29.8±24.24</td>
<td>25.6±25.91</td>
<td>0.267</td>
</tr>
<tr>
<td><strong>AST/ALT (%)</strong></td>
<td>1.2±0.78</td>
<td>1.0±0.39</td>
<td>0.088</td>
</tr>
<tr>
<td><strong>CK (U/L)</strong></td>
<td>710.9±787.34</td>
<td>83.3±44.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>LDH (U/L)</strong></td>
<td>320.9±150.80</td>
<td>160.3±41.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>TB (µmol/L)</strong></td>
<td>11.7±4.37</td>
<td>15.2±2.97</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>UCB (µmol/L)</strong></td>
<td>9.4±4.23</td>
<td>10.8±2.50</td>
<td>0.013</td>
</tr>
<tr>
<td><strong>CU (µmol/L)</strong></td>
<td>2.6±1.23</td>
<td>4.5±1.95</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>MTT score</strong></td>
<td>35.1±7.96</td>
<td>——</td>
<td>——</td>
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</table>
A comparison of demographics and laboratory data between PM patients and healthy individuals are shown in Table I. The serum concentrations of ALT, AST, CK and LDH, as muscle-derived enzyme, were higher in PM patients than healthy controls, and the serum concentrations of TB, CB and UCB were significantly lower in patients with PM compared with healthy controls, as shown in Figure 1 to 3.

Serum concentrations of TB were negatively correlated with ESR, CK and LDH in patients with PM (\( r=-0.494, p<0.001; r=-0.274, p=0.017; r=-0.282, p=0.014 \)). The results of analysis on the correlation between serum UCB and laboratory parameters showed that the serum concentration of UCB were negatively correlated with ESR and CK in PM patients (\( r=-0.424, p<0.001; r=-0.234, p=0.041 \)). Of note, the serum concentrations of TB and UCB were positively correlated with MMT score in patients with PM (\( r=0.328, p=0.004; r=0.333, p=0.004 \)).

Knowing that serum bilirubin levels may be influenced by gender, inflammatory states and liver function\(^7,20\). Thus, a multiple linear regression analysis was performed with adjustment for gender, ALT, AST, CRP and ESR in PM patients. Among patients with PM, serum concentrations of UCB were independently associated with MMT score in multiple linear regression analysis, as shown in Table II. No significantly correlation was found between serum concentrations of TB, CB and MMT score in PM patients in multiple linear regression analysis.

In the present study, we observed that the serum concentrations of TB, CB and UCB were significantly lower in patients with PM. Surprisingly, the serum concentrations of UCB were positively correlated with disease activity of PM patients in multiple linear regression analysis.

There is mounting evidence that lower serum concentrations of bilirubin have been observed in patients with coronary artery disease, stroke, metabolic syndrome and chronic kidney disease\(^22-24\). In previous studies, some investigators have demonstrated that lower serum bilirubin is associated with cardiovascular disease\(^25\). Others, like Kawamoto et al\(^26\) have found a reverse rela-
tionship between serum bilirubin and carotid atherosclerosis among elderly population. In addition, high serum bilirubin concentrations induced by infusion can attenuate bleomycin-induced pulmonary fibrosis by inhibiting lung inflammation. These previous researches may suggest a protective effect of serum bilirubin in some inflammation-linked diseases. On the other hand, interleukin-6, interleukin-10 and mortality score have been associated with lower bilirubin concentrations in patients with sepsis. A line of evidence attests that serum Ibil may be helpful to reduce immune stimulation. O’Malley et al in a prospective study observed an association between lower serum concentrations of UCB and smoking. In fact, UCB reacts with an oxidizing agent, and formed oxidized UCB consequent on excreting via the urine. In some autoimmune disorders, an underlying association between lower serum concentrations of bilirubin and Crohn’s disease has also been reported by Leniček et al. It has been demonstrated that serum bilirubin levels are negatively correlated with lupus disease activity, and a negatively association between bilirubin and ESR is well established in lupus patients. In our study, lower levels of bilirubin were suggested in PM patients, and UCB levels were negatively related to ESR and CK in patients with PM. These results may attribute to chronic inflammation in skeletal muscle of PM patients. Indeed, chronic skeletal muscle inflammation is a primary feature that results in muscle weakness in PM patients. Thus, we speculate that bilirubin as an endogenous antioxidant may be destroyed by chronic systemic inflammation, the lower bilirubin levels in PM patients may result from an over-consumption of bilirubin by inflammatory activity in patients with PM.

The present study, however, has some limitations that deserve a mention. First, the sample was relatively small since a lower prevalence of PM and relatively rigorous inclusive and exclusive criteria. Second, only single assessment method for disease activity was used in PM patients, other assessment method, such as muscle damage index, quantitative index of muscle and muscle injury index, should be considered in evaluating the relationship between bilirubin and disease activity in patients with PM. Finally, serum concentrations of bilirubin were not evaluated in treated patients with PM.

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Conclusions

We observed significantly lower serum concentrations of TB, CB and UCB in patients with PM, and suggested a potential association between serum concentrations of UBC and disease activity in PM patients.

Author Contributions

Yi-Fan Peng contributed to conceive the study, analyze the data, and draft the manuscript. Liang Zhang and Guo-Gang Pan contributed to collect data for the work. Ye-Sheng Wei confirmed the final version.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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