Original Article
The effects of β2-MG, RBP, and CRP on the diagnosis and prediction of the treatment outcomes of diabetic microangiopathy

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Received October 23, 2019; Accepted December 27, 2019; Epub March 15, 2020; Published March 30, 2020

Abstract: Objective: This study aims to investigate the diagnostic performance of serum high sensitivity C-reactive protein (CRP), urinary retinol-binding protein (RBP), and β2 microglobulin (β2-MG) for diabetic microangiopathy. Methods: A total of 146 patients with diabetes admitted to our hospital from February 2016 to February 2019 were selected as subjects. Among them, 76 patients with microvascular disease were included in the study group, and the other 70 patients without microvascular disease were enrolled in the control group. 5 mL of venous blood was collected before and after treatment, which was placed at room temperature for 30 min and centrifuged (4000 rpm/min) for 10 min to obtain upper serum. The serum β2-MG and RBP levels were measured using chemiluminescence and immunoturbidimetry, respectively, and the CRP levels were measured using a fully automated specialty protein analyzer. The clinical efficacy of the treatment was recorded. The diagnostic value of the combined RBP, β2-MG, and CRP for diabetic microangiopathy was analyzed using an ROC curve. The predictive value of combined RBP, β2-MG, and CRP for therapeutic outcomes was also analyzed using an ROC curve. The risk factors associated with diabetic microangiopathy were analyzed as well. Results: The serum levels of RBP, β2-MG, and CRP in the study group were significantly higher than they were in the control group (P<0.05), but after treatment, there were no significant differences in the serum levels of RBP, β2-MG, and CRP in both groups (P>0.05), and the RBP level was still higher than it was in the control group (P<0.05). After treatment, the serum levels of RBP, β2-MG, and CRP decreased in both groups compared with the levels before treatment (P<0.05). According to an ROC curve analysis, the diagnostic sensitivity for diabetic microangiopathy was 86.00%, and the specificity was 94.00% (P<0.001). The effective sensitivity for diabetic microangiopathy patients was 80.00%, and the specificity was 90.00% (P<0.001). A logistic regression analysis showed that RBP, β2-MG, and CRP are independent protective factors for diabetic microangiopathy (OR>1, P<0.05). Conclusion: The serum levels of β2-MG, RBP, and CRP in patients with diabetic microangiopathy are higher than they are in healthy populations. The combined measurement of β2-MG, RBP, and CRP has a good efficacy in the diagnosis and prediction of the treatment outcomes of diabetic microangiopathy.

Keywords: hs-CRP, RBP, β2-MG, diabetic microangiopathy

Introduction
Diabetes is one of the most common chronic diseases worldwide, and it has a very high incidence in many countries [1]. The survey results of Sharma et al. [2] showed that 406,344 cases of diabetes were found in 8,838,031 patients ranging in age from 0 to 99 years. The incidence was close to 5%. In recent years, with the continuous improvement of people’s living standards, the incidence of diabetes has increased yearly [3]. Due to the increasing numbers of patients with serious diabetes, the series of complications caused by diabetes has gradually become the primary challenge in clinical practice. There are many chronic diseases caused by diabetes, such as kidney disease, retinal disease, and bone disease [4]. Microangiopathy caused by diabetes cannot be ignored. Studies have shown that diabetic microangiopathy has gradually become the most common complication in diabetic patients [5]. Diabetic microangiopathy is relatively specific. The main pathological changes are a thick-
enning of the basement membrane accompanied by the deposition of transparent substances [6]. It is usually accompanied by different degrees of microcirculation abnormalities. Interaction with the basement membrane lesions will aggravate the progression and development of the disease [7]. Microangiopathy can occur in various tissues of the human body. Once microthrombus or a microvascular occlusion has formed, it will directly endanger the patient’s life and health [8]. Therefore, clinical researchers have been devoting themselves to exploring effective diagnostic and treatment methods for diabetic microangiopathy [9-11]. However, no remarkable progress has been achieved.

RBP and β2-MG are the newest indicators for clinically detecting kidney disease and are extremely sensitive to kidney damage [12]. At present, studies have shown that the measurement of the RBP and β2-MG levels is of great significance for the early screening of diabetic nephropathy and chronic renal failure, and RBP and β2-MG are closely related to the occurrence of microangiopathy [13, 14]. Therefore, we hypothesized that the measurement of the RBP and β2-MG levels could be used as an early screening indicator for diabetic microangiopathy. And in order to further improve the accuracy of the measurement, we also jointly measured the hypersensitive C-reactive protein (CRP) level, a commonly-used indicator in clinical practice and one that is extremely sensitive to the inflammatory response, which has important clinical implications for the future progress of treating diabetic microangiopathy. To confirm this, we carried out this experiment to determine the levels of RBP, β2-MG, and CRP in patients with diabetic microangiopathy and patients with simple diabetes, on which our experimental analysis was conducted, in order to provide an accurate and reliable reference for the future clinical diagnosis and treatment of diabetic microangiopathy.

Materials and methods

General information

A total of 146 patients with diabetes who were admitted to our hospital from February 2016 to February 2019 were selected as the study subjects. Among them, 76 patients with microvascular disease were included in the study group, and the other 70 patients without microvascular disease were enrolled in the control group. This experiment was approved by the Ethics Committee of our hospital, and all the above subjects signed an informed consent.

Inclusion and exclusion criteria

Inclusion criteria: Patients diagnosed with diabetes; patients meeting the diagnostic criteria for diabetic microangiopathy [15]; the patients in the study groups were diagnosed with diabetic microangiopathy using the urinary albumin excretion rate (UAER) and the Mogensen staging diagnosis [16] (3 UAER detections within no less than 6 months); the patients in the control group were diagnosed with diabetes but without diabetic microangiopathy; the patients received regular follow-ups and treatment in our hospital after diagnosis; patients with complete medical records; patients who agreed to cooperate with the medical staff in our hospital.

Exclusion criteria: Patients with other chronic diseases; patients with tumors; patients with organ failure; patients with severe hepatic insufficiency; patients complicated with renal disease; patients complicated with other inflammatory diseases; patients with other cerebrovascular diseases; patients with mental illness; patients with physical disabilities; patients with a drug allergy; patients who received chemoradiotherapy within 3 months before treatment; patients who were pregnant; patients unable to take care of themselves; patients with physical disabilities; patients transferred halfway.

Methods

The patients in the study group were given the conventional treatment of lowering their blood glucose and blood lipids. Calcium benzenesulfonate tablets (Jiangsu Wangao Pharmaceutical Co., Ltd., GYZZ H20080288) were taken 3 times a day. Pancreatic kininogenase enteric-coated tablets (Henan Lingyou Pharmaceutical Co., Ltd., GYZZ H41022915) were taken 3 times a day, at 500 mg/time and 12 U/time. The course of treatment was 2 months.

5 mL of fasting venous blood was obtained before and one month after treatment. The blood samples were placed at room tempera-
ture for 30 min and centrifuged (4000 rpm/min) for 10 min to obtain upper serum. Serum β2-MG levels (the kit was purchased from Shanghai Xinyu Biotechnology Co., Ltd., xy-CL-R0699c) were measured using chemiluminescence. Serum RBP levels (the kit was purchased from Shanghai Xinfan Biotechnology Co., Ltd., XF110-a) were measured using immunoturbidimetry. The detection process was carried out in strict accordance with the kit’s instructions. The CRP levels were measured using a fully automated specialty protein analyzer (Beckman Coulter IMMAGE 800). At the end of the 2-month treatment, according to Lehrke [17], the clinical efficacy of the treatment was classified into markedly effective, effective, and ineffective.

Outcome measures

Primary indicators: The RBP, β2-MG, and CRP levels of the study group before and after treatment, and the predictive value of RBP, β2-MG, and CRP in the diagnosis diabetic patients with microangiopathy.

Secondary indicators: The predictive value of RBP, β2-MG, and CRP in the clinical effect of diabetic microangiopathy before and after treatment in the study group, and the analysis of the related risk factors of microangiopathy in diabetic patients.

Statistical methods

SPSS 24.0 statistical software was used for the statistical analysis of all the experimental results (Beijing Strong Vinda Information Technology Co., Ltd.). All the graphs were drawn using GraphPad 8 software (Shenzhen Tianruoji Software Technology Co., Ltd.), and the results were confirmed by a secondary check. The enumeration data such as patient gender were expressed as rates and were compared between groups using the chi-square tests; the measurement data such as RBP, β2-MG, and CRP were expressed as the means ± standard deviations, and independent t-tests were used for the comparisons between groups. To measure the diagnostic performance and predicting values of combined RBP, β2-MG, and CRP, an SPSS binary multi-variate logistic regression model was set up respectively by including RBP, β2-MG, and CRP as independent variables and then an ROC was performed. Those significant variables were analyzed using a multivariable logistic regression analysis to seek the independent predictors for diabetic microangiopathy. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. P<0.05 was considered statistically significant.

Results

Comparison of the general data between the two groups of patients

The patients in the two groups had no significant differences in terms of age, BMI, white blood cells (WBC), red blood cells (RBC), platelets (PLT), gender, marital status, education, ethnicity, place of residence, smoking, exercise, family medical history, or eating habits (P>0.05).

The patients in the study group had significantly higher fasting blood glucose levels, total cholesterol, and rates of microvascular disease, and lower insulin than the patients in the control group (P<0.001) (Table 1).

Comparison of the RBP, β2-MG, and CRP concentrations

The RBP, β2-MG, and CRP serum levels in the study group were significantly higher than in the control group (P<0.05), but after the treatment, there were no significant differences in the serum levels of RBP, β2-MG, and CRP in both groups (P>0.05), and the RBP level was still higher in the study group than it was in the control group (P<0.05). After treatment, the serum levels of RBP, β2-MG, and CRP decreased in both groups compared with the levels before treatment (P<0.05) (Figure 1).

Combined RBP, β2-MG, and CRP for diagnosing diabetic microangiopathy

According to the ROC curve analysis, when the cut-off value was 14.08, the sensitivity and specificity of RBP for the diagnosis of microangiopathy were 92.00% and 62.00%, respectively. When the cut-off value was 1.77, the sensitivity and specificity of β2-MG for the diagnosis of microangiopathy were 98.00% and 44.00%, respectively. When the cut-off value was 7.55, the sensitivity and specificity of CRP for the diagnosis of microangiopathy were 92.00% and 58.00%, respectively. A binary logistic regression analysis was performed with RBP, β2-MG,
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and CRP as independent variables. Three joint prediction models were obtained, Logit (P) = 10.762 + -0.820β2-MG + -0.849CRP + -0.358RBP. When the cut-off value was 0.68, the sensitivity and specificity of the model for the diagnosis of microangiopathy in diabetic patients were 86.00% and 94.00%, respectively (Table 2; Figure 2).

Combined RBP, β2-MG, and CRP for predicting diabetic microangiopathy treatment outcomes in the study group

Of the 76 patients with diabetic microangiopathy, 24 cases were significantly effective, 36 cases were effective, and 16 cases were ineffective. The patients with significant and effec-
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The relationship between the RBP, β2-MG, and CRP levels and diabetic microangiopathy

The variables with differences in the above results were assigned (the assignment table is shown in Table 3), and then forward LR was selected to perform a multi-factor logistic regression analysis. The results showed that RBP was an independent protective factor for diabetic microangiopathy (OR: 1.712; 95% CI: 3.268–7.684; P=0.024). β2-MG was an independent protective factor for diabetic microangiopathy (OR: 1.094; 95% CI: 0.924–1.872; P<0.001). CRP was an independent protective factor for diabetic microangiopathy (OR: 1.923; 95% CI: 2.541–7.213; P=0.006). Fast blood glucose was an independent protective factor for diabetic microangiopathy (OR: 0.662; 95% CI: 1.031–4.682; P=0.014). Insulin was an
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Discussion

Diabetic microangiopathy has a great impact on the various functions and organs of a patient’s body. It may also cause a variety of complications, such as hypertension, myocardial infarction, kidney disease, and microaneurysms [18]. At present, the incidence of diabetes increases as one ages, and microangiopathy has attracted more and more clinical attention. Such chronic diseases should be detected and treated early to improve the clinical efficacy of the patients’ treatment, reduce the possibility of organ damage, and ensure patient safety [19].

At present, the treatment of microangiopathy has been widely recognized in clinical practice. The drug combination of calcium benzenesulfonate tablets and pancreatic kininogenase enteric-coated tablets can reduce blood sugar, effectively dilate blood vessels, and prevent the occurrence of microthrombus and occlusion [20, 21]. As microangiopathy is latent at the early stage, it is usually impossible to screen for it when it first occurs. Jansen et al. [22] suggested that diabetic microangiopathy can be effectively screened by detecting the infiltration of endothelial microparticles into the microRNA. Zhou et al. [23] showed that miR-22-3p has affected the occurrence of diabetic microangiopathy by up-regulating DAPK2. However, these indicators are more complicated and are not conducive to widespread clinical application. Therefore, for diabetic microangiopathy, a detection indicator with a convenient detection method, a high sensitivity and a strong specificity is important for the early screening of microangiopathy. The subsequent rehabilitation of patients can be evaluated for treatment in real time. At present, there are still few studies on this subject at home and abroad. In this study, the screening was carried out according to rigorous inclusion and exclusion criteria. Excellent experimental instruments and reagents were used. The experiments have effectively validated the clinical significance of RBP, β2-MG, and CRP for the diagnosis and prediction of the treatment outcomes of diabetic microangiopathy.

The results of this experiment showed that the serum RBP, β2-MG, and CRP concentrations of patients with diabetic microangiopathy in the study group were higher than those in the control group. It indicated that RBP, β2-MG, and CRP may be involved in the development and progression of diabetic microangiopathy. Such results are consistent with the study of Wu et al. [24], which discovered higher urinary RBP levels in patients with Type II diabetes and the study of Wang et al. [25] which reported higher β2-MG levels in diabetic patients.

These two studies confirm the results of this study. The ROC test showed that the combined measurement of the RBP, β2-MG, and CRP levels has a good predictive value for the diagnosis and treatment of microangiopathy. It indicated that RBP, β2-MG, and CRP can be effec-
Table 4. Multi-variate logistic regression analysis

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>S.E.</th>
<th>Wals</th>
<th>Sig.</th>
<th>Exp (B)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBP</td>
<td>1.712</td>
<td>0.812</td>
<td>5.632</td>
<td>0.024</td>
<td>5.277</td>
<td>3.268~7.684</td>
</tr>
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<td>β2-MG</td>
<td>1.094</td>
<td>0.038</td>
<td>9.54</td>
<td>&lt;0.001</td>
<td>1.084</td>
<td>0.924~1.872</td>
</tr>
<tr>
<td>CRP</td>
<td>1.923</td>
<td>0.580</td>
<td>11.834</td>
<td>0.006</td>
<td>6.521</td>
<td>2.541~7.213</td>
</tr>
<tr>
<td>Fasting blood sugar</td>
<td>0.662</td>
<td>0.562</td>
<td>8.552</td>
<td>0.014</td>
<td>2.872</td>
<td>1.031~4.682</td>
</tr>
<tr>
<td>Insulin</td>
<td>1.436</td>
<td>0.468</td>
<td>9.214</td>
<td>0.027</td>
<td>5.147</td>
<td>2.628~6.324</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.042</td>
<td>0.012</td>
<td>12.241</td>
<td>&lt;0.001</td>
<td>0.884</td>
<td>0.801~0.939</td>
</tr>
<tr>
<td>Courses of disease</td>
<td>0.024</td>
<td>0.015</td>
<td>6.812</td>
<td>0.005</td>
<td>1.012</td>
<td>0.987~1.028</td>
</tr>
<tr>
<td>Family history</td>
<td>0.033</td>
<td>0.014</td>
<td>6.824</td>
<td>0.015</td>
<td>1.024</td>
<td>1.002~1.089</td>
</tr>
</tbody>
</table>

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does not rule out that the renal function of the patient can be affected by the diabetes. If the renal function declines, the β2-MG concentration will rise more significantly. CRP is an excellent sensitive indicator of the vascular inflammatory response and a non-specific marker of systemic inflammation [30]. At present, the mechanism of action of CRP in diabetes has been initially confirmed. It is agreed at home and abroad that CRP mainly ingests low-density lipoprotein by inducing macrophages, and forms cytokines and tissue factors that can adhere to the vascular endothelium. The function of the vascular endothelium is damaged [31, 32]. In microangiopathy, the mechanism of action should be similar. The massive accumulation of CRP in microvessels forms microvascular tissue and thrombus, which seriously affects the progress of the entire microvascular cycle. This is precisely related to the mechanism of action of our β2-MG. From the observation of the above results, we found that single detections of β2-MG, RBP, and CRP are more sensitive to the diagnosis of microangiopathy in diabetic patients, but the specificity is very low. This is also in line with the current research status of the three factors in the clinic, which can reflect the significant changes in the inflammatory, injury, and necrosis that occur in the patient’s body but cannot accurately reflect the pathological injuries that the patients experience. By a combination of determining the three serum levels, the specificity of the microangiopathy can be up to 94.00%, which is of great significance for the early screening of diabetic patients with microangiopathy in the future. At present, the diagnosis of microangiopathy in clinics is mainly performed by imaging technology, and some of the hidden and complicated microvessels are not carefully observed. Moreover, the judgment of the result depends on the subjective consciousness of the doctor who reads the image, and there may be certain judgment errors. Through the detection of blood markers, the results are objective, and it is convenient to obtain samples, which greatly reduces the cost, time, and the possibil-
ity of misdiagnosis. In addition, blood samples can be kept for a long period, which is convenient for clinical reexamination at any time. This is also more conducive for helping clinicians understand the changes in patients’ conditions over time.

However, our conjecture still needs to be verified by further research. Since there are few studies on the effects of β2-MG and RBP in diabetic patients with microangiopathy, we still cannot understand the mechanism of its pathogenesis. The results of the experiment cannot be compared with the relevant similar studies. Also, the short experiment time made it impossible to judge the influence of β2-MG, RBP, and CRP on patient prognosis. Moreover, there are still many potential cytokines or inflammatory factors to be discovered that may affect the progression of diabetic microangiopathy, so chances are that some other indicators for diabetic microangiopathy may be more sensitive and effective than the three indicators in this experiment. We will conduct a longer follow-up study and further expand the sample size. In order to verify our conjecture, we will perform an in-depth study on the effects of β2-MG, RBP, and CRP on diabetic microangiopathy and determine the exact mechanisms of β2-MG, RBP, and CRP as early as possible.

In summary, the serum levels of β2-MG, RBP, and CRP in patients with diabetic microangiopathy are higher than those in the normal population. The combined determination of the levels of β2-MG, RBP, and CRP has a good efficacy in the diagnosis and treatment of diabetic microangiopathy.

Disclosure of conflict of interest

None.

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