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Original Article

Levels of serum superoxide dismutase and high sensitive C-reactive protein in type 2 diabetic patients with lower extremity vascular disease are enhanced by interventional treatment

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Abstract: Objectives: This study is to determine the levels of serum superoxide dismutase (SOD) and high sensitive C-reactive protein (hs-CRP) in type 2 diabetic patients with lower extremity vascular disease before and after interventional treatment. Methods: A total of 65 patients were enrolled in this study, including 35 mails and 30 females. Another 65 healthy individuals were used as control, including 41 males and 24 females. Lesions and degrees of stenosis were determined by computed tomography angiography. Contralateral iliac artery and proximal femoral artery occlusion were treated by retrograde femoral artery puncture. The levels of serum SOD and hs-CRP were determined by enzyme-linked immunosorbent assay. Correlation was analyzed by Pearson's test. Progression-free survival curve was analyzed by Kaplan-Meier method. Results: The levels of serum SOD at 20 min, 24 hr, 7 d, and 14 d after surgery were significantly decreased compared with those before surgery ($P < 0.05$). The levels of serum hs-CRP at 20 min and 24 hr after surgery were increased compared with those before surgery ($P < 0.05$). The level of serum hs-CRP at 14 d after surgery was significantly lower than that before surgery ($P < 0.05$). The correlation between SOD and hs-CRP was positive before surgery ($r = 0.03$, $P < 0.001$), but negative at 24 hr after surgery ($r = -0.008$, $P < 0.001$). The levels of serum SOD were significantly lower than median value ($P < 0.05$), while the Levels of serum hs-CRP were significantly higher than median value ($P < 0.05$). Conclusions: The levels of serum SOD and hs-CRP were significantly different before and after interventional treatment. The levels of serum SOD and hs-CRP can be used as indicators for the efficacy and prognosis of interventional treatment on type 2 diabetic patients with lower extremity vascular disease.

Keywords: Type 2 diabetes, lower extremity vascular disease, interventional treatment, superoxide dismutase, high sensitivity C-reactive protein

Introduction

Diabetes is one of the diseases that seriously threaten human health [1]. Lower extremity vascular disease is one of the common serious diabetes complications, with increasing incidence year by year. Most studies on lower extremity vascular disease are focused on the detection of relevant factors in the blood [2, 3]. The main pathological change that occurs in diabetes with lower extremity vascular disease is atherosclerosis. It is reported that increased reactive oxygen products and decreased antioxidants play important roles in atherosclerosis formation [4]. For example, the levels of serum superoxide dismutase (SOD) and high sensitive C-reactive protein (hs-CRP) are key to type 2 diabetes with lower extremity vascular disease [5, 6]. As an antioxidant, SOD mainly exists in cell metabolism, playing a protective role in cells by the disproportionation of toxic free radicals to inactive hydrogen peroxide and oxygen molecules [7, 8]. Elevated blood sugar can enhance the levels of glycosylated proteins. Self-oxidation of sugar and glycosylated proteins increases the levels of free radicals in diabetic patients, inhibiting antioxidant capacity [5]. Diabetes-induced oxidative stress causes damages to endothelial cells [9]. Therefore, SOD plays an important role in systemic vascu-

Table 1. General information of patients

General information	Treatment group (n = 65)	Control group (n = 65)	*P* value
Age (years)			
> 30 or < 70	48.63 ± 12.67*	43.61 ± 8.17	< 0.05
Gender			
Male/female	35/30	41/24	No significance
Smoking history (years)			
> 20 or < 30	56	43	No significance
Lower extremity arterial disease			
Lilac, femoral, popliteal artery/tibial, peroneal artery	31/39	0/0	No significance
Biochemical indicators			
LDL-C (mM)	3.95 ± 1.18*	3.25 ± 0.82	< 0.05
TG (mM)	2.42 ± 1.53*	1.95 ± 1.22	< 0.05
TC (mM)	5.15 ± 1.84*	4.23 ± 1.37	< 0.05
BMI (kg/m^2)			
> 24.0 or < 23.9	29.44 ± 4.19*	27.16 ± 2.91	< 0.05

Note: LDL-C, low-density lipoprotein-cholesterol; TG, triglyceride; TC, total cholesterol. Data were represented as means ± standard deviation. *$P < 0.05$ compared with control.

lar disease. As a marker of inflammation, hs-CRP plays important roles in the occurrence and development of diabetes-related vascular diseases. There are many studies about changes in serum antioxidant capacity index and inflammatory indicators, which are mainly focused on SOD and hs-CRP in the serum that is different from those of healthy individuals [10, 11].

As a minimally invasive technique, interventional treatment is safe, effective, and reproducible, and is thus used as the preferred treatment for lower extremity vascular disease [12]. Interventional treatment opens arteries of lower extremity stenosis or occlusion, and restores blood flow to foot in lower limb ischemic lesion [13]. However, there are few reports about the levels and correlation of serum SOD and hs-CRP before and after surgery. In this study, we detected the levels of serum SOD and hs-CRP in type 2 diabetic patients with lower extremity vascular disease before and after interventional treatments.

Materials and methods

Patients

A total of 65 patients were enrolled in this study, including 35 males and 30 females. The age of the patients ranged from 30 to 70 years, with an average age of 48.63 ± 12.67 years. For control, 65 healthy individuals (41 males and 24 females, with an average age of 43.61 ± 8.17 years, ranging from 30 to 72 years) were enrolled. All patients had varying degrees of clinical manifestations, including 53 cases of intermittent claudication, 49 cases of rest pain, 16 cases of changed skin color, and 28 cases of weakened dorsalis pedis artery pulse.

The inclusion criteria were as follows: i) patients met the diagnostic criteria defined by World Health Organization in 1999; ii) patients had diabetes-related vascular diseases such as cerebral artery, carotid, lower limb arteries, and other vascular atherosclerosis. The exclusion criteria were as follows: patients suffered from infectious diseases, cancer, heart failure, severe liver and kidney dysfunction, acute cerebral infarction, or ketoacidosis. Prior written informed consent was obtained from all patients. The study was approved by the Ethics Committee of Baotou Medical College.

Surgery

Lesions and degrees of stenosis were determined by computed tomography angiography. Contralateral iliac artery and proximal femoral artery occlusion were treated by retrograde femoral artery puncture. Midst-inferior segment of ipsilateral femoral artery, popliteal artery, and leg artery occlusion were treated by

Table 2. Levels of SOD and hs-CRP in serum

Group	SOD (U/ml)	*P* value	hs-CRP (pg/ml)	*P* value
Control	19.81 ± 8.14		2.85 ± 1.17	
Therapy				
Before surgery	31.56 ± 9.43*	< 0.05	5.85 ± 2.18*	< 0.05
20 min after surgery	29.45 ± 12.14#	< 0.05	16.27 ± 9.31#	< 0.05
24 hr after surgery	28.22 ± 7.53#	< 0.05	45.75 ± 12.63#	< 0.05
7 d after surgery	23.72 ± 13.12#	< 0.05	6.27 ± 4.63	> 0.05
14 d after surgery	28.72 ± 6.78#	< 0.05	4.35 ± 1.61#	< 0.05

Note: SOD, superoxide dismutase; hs-CRP, high sensitive C-reactive protein. Data were represented as means ± SD. *$P < 0.05$ compared with control. #$P < 0.05$ compared with pre-operation group.

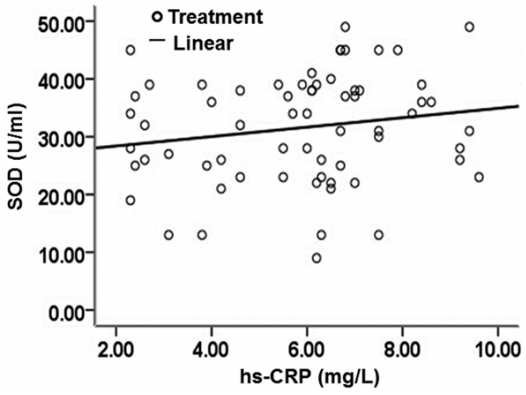

Figure 1. Correlation between serum SOD and hs-CRP before surgery. Enzyme-linked immunosorbent assay was performed to detect the levels of serum SOD and hs-CRP before surgery. Pearson's correlation analysis was performed to analyze the correlation between serum SOD and hs-CRP before surgery.

antegrade femoral artery puncture. After successful puncture, patients were implanted with arterial sheath, and given systemic heparin. Then, patients were administered with contrast agent for imaging in limb arteries. Finally, balloon angioplasty and stent implantation were performed with dedicated guide wires, catheters, and other equipments for stenosis.

Enzyme-linked immunosorbent assay (ELISA)

A total of 3 ml fasting, peripheral venous blood was collected before surgery and at 20 minutes, 24 hours, 7 days, 14 days and 12 months after surgery. Blood was collected in sterile tubes, and left standing for 30 minutes, followed by centrifugation at 5000 rpm for 5 minutes. Then, the levels of serum SOD and hs-CRP were detected by enzyme-linked immunosorbent assay.

Statistical analysis

All data were analyzed by SPSS 20.0 software (IBM, USA). Data were represented as percentage, and expressed as means ± standard deviation. To compare differences between groups, *t*-test was used. Correlation was analyzed by Pearson's test. Progression-free survival curve was analyzed by Kaplan-Meier method. *P* value less than 0.05 was considered statistically significant.

Results

Age, BMI, and biochemical indicators of type 2 diabetic patients with lower extremity arterial disease are different from those of healthy individuals

General information of 65 type 2 diabetic patients with lower extremity arterial disease showed different degrees of stenosis or occlusion in iliac, femoral, popliteal, tibial, and peroneal artery of the patients (**Table 1**). Age, body mass index (BMI), and biochemical indicators in the treatment group were significantly higher than those in the control group (Age: 48.63 ± 12.67 vs 43.61 ± 8.17 years; BMI: 29.44 ± 4.19 vs 27.16 ± 2.91 kg/m^2; low-density lipoprotein-cholesterol: 3.95 ± 1.18 vs 3.25 ± 0.82 mmol/L; triglyceride: 2.42 ± 1.53 vs 1.95 ± 1.22 mmol/L; total cholesterol: 5.15 ± 1.84 vs 4.23 ± 1.37 mmol/L) ($P < 0.05$). These results indicated that differences existed in age, BMI, and biochemical indicators between type 2 diabetic patients with lower extremity arterial disease and healthy individuals.

Levels of serum SOD and hs-CRP in type 2 diabetic patients with lower extremity vascular disease are enhanced by interventional treatments

To determine the levels of serum SOD and hs-CRP in type 2 diabetic patients with lower extremity vascular disease before and after interventional treatments, ELISA was performed. ELISA results showed that the levels of serum SOD and hs-CRP before surgery were significantly higher than those in the control

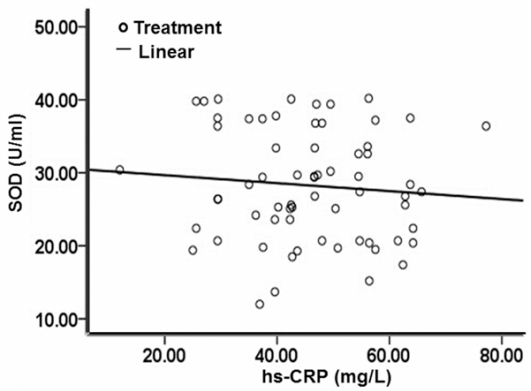

Figure 2. Correlation between serum SOD and hs-CRP after surgery. Enzyme-linked immunosorbent assay was performed to detect the levels of serum SOD and hs-CRP at 24 hr after surgery. Pearson's correlation analysis was performed to analyze the correlation between serum SOD and hs-CRP at 24 hr after surgery.

group ($P < 0.05$). In addition, the levels of serum SOD at 20 min, 24 hr, and 7 d after surgery were significantly higher than those before surgery ($P < 0.05$). The level of serum SOD was increased at 14 d after surgery, but was significantly lower than that before surgery ($P < 0.05$). The levels of serum hs-CRP at 20 min and 24 hr after surgery were significantly higher than those before surgery ($P < 0.05$), but was decreased at 7 d after surgery. Of note, the level of serum hs-CRP at 14 d after surgery was significantly lower than that before surgery ($P < 0.05$) (**Table 2**). These results suggested that the levels of serum SOD and hs-CRP before surgery were significantly higher than those in the control group.

Positive correlation between the levels of serum SOD and hs-CRP before interventional treatment is altered after surgery

To determine the correlation between serum SOD and hs-CRP in type 2 diabetic patients with lower extremity vascular disease before and after interventional treatment, Pearson's correlation analysis was performed. The levels of serum SOD and hs-CRP were positively correlated before surgery ($r = 0.03$, $P < 0.001$) (**Figure 1**), but were negatively correlated at 24 hr after surgery ($r = -0.008$, $P < 0.001$) (**Figure 2**). These results indicated that positive correlation observed between the levels of serum SOD and the levels of hs-CRP before surgery was altered at 24 hr after surgery.

Levels of serum SOD and hs-CRP after surgery have a great impact on the prognosis of type 2 diabetic patients with lower extremity vascular disease

To determine the effects of serum SOD and hs-CRP on the prognosis of type 2 diabetic patients with lower extremity vascular disease, Kanplan-Meier analysis was used. Follow-up of type 2 diabetic patients with lower extremity vascular disease for 12 months after interventional treatment showed that median values of SOD and hs-CRP were 5.85 U/ml and 31.56 pg/ml, respectively, as determined by the levels of serum SOD and hs-CRP before surgery (**Figure 3**). The prognosis was poor, and disease progression was demonstrated by stenosis or occlusion in lower extremity veins. These results indicated that the levels of serum SOD and hs-CRP after surgery had a great impact on the prognosis of type 2 diabetic patients with lower extremity vascular disease.

Discussion

Diabetes is a disease of abnormal metabolism of glucose, lipid, and protein, in which pancreatic islet dysfunction is caused by complex and multiple factors. Type 2 diabetes with lower extremity vascular disease is one of the complications of diabetes. SOD is an oxygen free radical scavenger [14], the levels of which are decreased by long-term mechanical stimuli of sustained hyperglycemia in lower extremity veins. Therefore, the ability of scavenging oxygen free radicals is decreased, resulting in vascular stenosis by plaques. As a sensitive, nonspecific marker of inflammation, hs-CRP exists in the occurrence and development of atherosclerosis [15]. It is very important to determine the levels of hs-CRP before and after surgery for anti-inflammation and restenosis.

In the present study, the levels of serum hs-CRP and SOD in type 2 diabetic patients with lower extremity vascular disease were determined before and 20 minutes, 24 hour, 7 days or 14 days after interventional treatment. ELISA results showed that the levels of serum SOD were decreased in a short period of time after interventional treatment, indicating enhanced oxidative stress response. The levels of serum SOD were increased at 14 d after surgery. Interventional treatment on lower extremity arterial stenosis caused transient mechanical

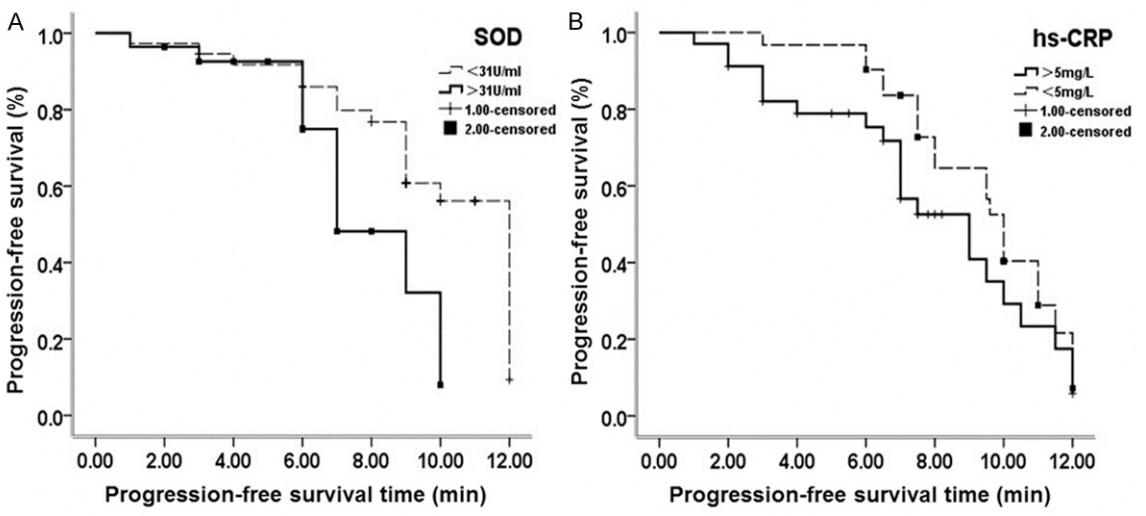

Figure 3. Effects of serum (A) SOD and (B) hs-CRP on progression-free survival time after surgery. Kanplan-Meier analysis was performed to analyze the effects of serum SOD and hs-CRP. Type 2 diabetic patients with lower extremity vascular disease were followed up for 12 months after interventional treatment. Stenosis or occlusion in lower extremity vascular was considered as disease progression. Median values of SOD and hs-CRP were determined by the levels of serum SOD and hs-CRP before surgery.

injury in vascular endothelial cells and reperfusion injury in endangium, leading to enhanced damage responses to lipid peroxides. Then, a great amount of SOD was consumed and lower extremity vascular oxidative stress response was weakened. Finally, vascular endothelia were injured. Therefore, restenosis after surgery was significantly associated with oxidative stress, which was one of the independent risk factors for vascular restenosis. The levels of serum hs-CRP at 20 min and 24 hr after surgery were significantly increased compared with those before surgery. All these results indicated that inflammation was increased in the early period after surgery, leading to restenosis after interventional treatment. The levels of serum hs-CRP at 14 d after surgery were significantly decreased compared with those before surgery, indicating that inflammatory responses were weakened.

In the present study, correlation between serum SOD and hs-CRP was detected before and after interventional treatment. Positive correlation of the two factors was shown before surgery. All these results indicated that diabetes decreased serum SOD levels, reduced antioxidant capacity, and damaged vascular endothelial cells, leading to the final formation of plaques. Negative correlation of serum SOD and hs-CRP was found at 24 hr after surgery. Angioplasty is the main method of interventional treatment for vascular recanalization. Despite balloon dilatation or stent placement, original plaques always existed. Antioxidant capacity of vascular endothelial cells was decreased in a short time. Moreover, endangium was easily torn in the process of angioplasty, resulting in inflammatory response in lower extremity veins, which was increased in the short term. Results of Kanplan-Meier analysis showed that the level of serum SOD was lower than median value, while the level of serum hs-CRP was higher than median value. Poor prognosis indicated that the levels of serum SOD and hs-CRP after surgery had a great impact on the prognosis of type 2 diabetic patients with lower extremity vascular disease. In conclusion, serum SOD and hs-CRP were highly specific factors in type 2 diabetic patients with lower extremity vascular disease. The levels of serum SOD and hs-CRP could be used as indicators for the efficacy and prognosis of interventional treatment on type 2 diabetic patients with lower extremity vascular disease.

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Disclosure of conflict of interest

None.

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