The clinical evaluation of combined detection of microcirculation, lipid metabolism, and inflammatory-related factors in the treatment of diabetic nephropathy with atorvastatin

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Abstract
This study was designed to analyze the effects of atorvastatin on microcirculation, blood lipids, inflammatory factors, and characteristic markers in patients with diabetic nephropathy. A total of 170 patients with diabetic nephropathy randomly divided into control and study groups with 85 patients in each group. The control group was treated with diet and lifestyle intervention, and hypoglycemic drugs. The study group was additionally treated with atorvastatin. Nitric oxide (NO), endothelin-1 (ET-1), thromboxane-2 (TXB2), 6-ketone-prostaglandin F-1α (6-Keto-PGF-1α), superoxide dismutase (SOD), total cholesterol (TC), triacylglycerols (TGs), low-density lipoprotein (LDL), high-density lipoprotein (HDL), C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), homocysteine (Hcy), cystatin C (CysC), and vascular endothelial growth factor (VEGF) levels were observed for 8 weeks. Post-treatment of atorvastatin, the levels of NO, 6-Keto-PGF-1α, and SOD were significantly higher than pre-treatment in both groups, while the levels of ET-1 and TXB2 were lower than pre-treatment (P < 0.05). The levels of NO, 6-Keto-PGF-1α, and SOD in the study group post-treatment were significantly higher (P < 0.05) than the control group, and the levels of ET-1 and TXB2 in the study group were lower than the control group. After 8 weeks, the levels of TC, TG, and LDL were significantly lower, while the level of HDL was significantly higher in the study group. The level of TC was lower in the control group of post-treatment, while the HDL level was higher than pre-treatment (P < 0.05). The levels of CRP, TNF-α, and IL-6 in the study group of post-treatment were significantly lower than pre-treatment comparing to the control group (P < 0.05). There was no statistical significance (P > 0.05) for above-mentioned indicators in control groups of pre- and post-treatment. The levels of VEGF, CysC, and Hcy in the two groups were lower than pre-treatment. Atorvastatin could effectively improve all the study parameters.

Keywords
atorvastatin, inflammatory factors, microcirculation, nephropathy

Introduction
Diabetic nephropathy (DN) is not only one of the major microvascular complications of diabetes but also one of the important causes of chronic renal failure. The pathological manifestations of diabetes include basement membrane thickening, glomerular hypertrophy and extracellular matrix accumulation, and then develop interstitial fibrosis.
and glomerulosclerosis.\textsuperscript{2} With the increase in the diabetic patients in recent years, the incidence of DN also increases, which brings adverse impact on the patients’ health and quality of life. The main causes of DN are genetic factors, metabolic and hemodynamic effects, functional changes in glomerular filtration barrier, non-enzymatic glycosylation of proteins, increased polyol channel activity and inositol metabolism disorders, hormones, and cytokines.\textsuperscript{3} A study found that the blood glucose, total cholesterol (TC), homocysteine (Hcy), C-reactive protein (CRP), blood pressure, and DN were closely related.\textsuperscript{4} The statins have good lipid-lowering and anti-oxidant effects, which could reduce the cholesterol synthesis by inhibiting the activity of hydroxymethyl-diacyl-CoA reductase, thus further playing the role of lipid regulation. Some clinical findings show that the lipid-lowering regimen could effectively delay the occurrence and development of DN with good safety in the use of statins.\textsuperscript{5} In order to make better use of the statins to improve the renal function impairment, the atorvastatin was used in our hospital to treat 85 patients with DN, and the curative effect was found to be significant. Combined with clinical practice, we also checked the effects of atorvastatin on biochemical indicators, blood sugar, blood pressure, and inflammatory factors in order to disclose the application of atorvastatin in the treatment of DN.

Material and methods

General information

In total, 170 patients with DN who hospitalized from January 2016 to July 2017 in our hospital (Haiyang People’s Hospital, Haiyang, Shandong, China) were enrolled. All of the patients were local and eligible for the diagnostic criteria of Expert Consensus on prevention and treatment of Diabetic Nephropathy (2014 Edition). The relevant diagnostic criteria were as follows: 90 men and 80 women; age ranged from 46 to 79 years with mean age of 56.3 ± 5.4 years; body mass index was 21–27 kg/m\textsuperscript{2}, with average of 22.6 ± 2.5 kg/m\textsuperscript{2}; the duration of diabetes was from 6 to 15 years with an average duration of 9.5 ± 2.3 years; the duration of DN was from 6 to 37 months, with an average of 17.5 ± 2.4 months; the stage of DN: 105 cases in stage III and 65 cases in stage IV. The selection criteria are as follows:

1. Patients meet the relevant diagnostic criteria of Diabetic Nephropathy Experts Consensus (2014 Edition);
2. Patients were with stable blood glucose levels;
3. Patients have a good understanding, clear consciousness, and could cooperate with the examination and treatment;
4. The patients and/or family members signed the informed consent.

The exclusion criteria are as follows:

1. Other causes of kidney impairment, such as hypertension, glomerulonephritis, infection, and obstruction;
2. Patients had poor control of blood glucose levels and even had ketoacidosis;
3. Severe heart, liver, and other organs dysfunction;
4. Patients with malignant tumors;
5. Currently using angiotensin receptor blockers (ARBs) or anticoagulant drugs;
6. Patients allergic to the components of the drug undergoing treatment;
7. Pregnant women or lactating women;
8. Patients and/or family members disagree with the treatment.

The selected patients were randomly divided into control and study groups, with 85 cases in each group (n = 85). There were no statistically significant differences in sex ratio, age, body mass index, duration of diabetes mellitus, duration of DN, and stage of disorder.

The treatment methods

The control group using diet and lifestyle interventions, oral hypoglycemic drugs to control blood sugar, and other conventional treatments: (1) diet and lifestyle: a low protein diet, which could reduce the proteinuria, improving complications of DN and delay renal function. Following with moderate exercise, could quit smoking and alcohol drinking. (2) Blood glucose: the application of insulin, the stimulation of pancreatic β-cell drugs, the insulin sensitizers, and α-glucosidase inhibitor undergoing combination
Among these, the biguanide could cause lactic acidosis; thiazolidinediones may lead to edema; exacerbation of congestive heart failure, need to be used with careful consideration; and the glucosidase inhibitors mainly metabolized by the kidneys, the patients with severely impaired renal function should be used very carefully. The patient’s blood glucose level should be monitored in real time, and the hypoglycemic agents should be adjusted for dosage or drugs, to effectively control the glycosylated hemoglobin level at 7% to 9%. (3) Anti-hypertension: hypertension is one of the main reasons for the occurrence and development of DN, for which angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) drugs are preferred as first choice. Carry out continuous treatment for 8 weeks.

The study group was additionally given atorvastatin calcium tablets (Lipitor®; Pfizer Pharmaceutical Co., Ltd): 10 mg/d on the basis of the control group; the dose was increased as per the progress in the treatment; however, the maximum dose was 40 mg/d, for 8 weeks.

**Observe the indicators**

Blood (5 mL) was drawn from the elbow vein early in the morning from the patients of both groups. It was subjected to ethylenediaminetetraacetic acid (EDTA)-2Na anticoagulant treatment, centrifuged at 1500 r/min for 10 min, and the supernatant was discarded.

**Microcirculation index detection**

The indexes of microcirculation, that is, the levels of serum nitric oxide (NO), endothelin-1 (ET-1), thromboxane-2 (TXB2), 6-ketone-prostaglandin F-1α (6-Keto-PGF-1α), and superoxide dismutase (SOD) were detected by enzyme-linked immunosorbent assay (ELISA) before and after treatment. The Kits were purchased from Shanghai Tong Wei Industrial Co., Ltd, following strict instructions for operation. All procedures were carried out in triplicates.

**The lipid detection**

The levels of serum TC, triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were measured by Roche Modular automatic biochemical analyzer.

**Inflammatory factor test**

Serum CRP, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) levels were measured pre- and post-treatment with ELISA.

**The detection of characteristic markers**

Hcy and cystatin C (CysC) were detected with the help of Hitachi 7080 automatic biochemical analyzer. The vascular endothelial growth factor (VEGF) was detected by Bio-Rad 850 enzyme standard analyzer.

**Ethical consideration**

This study was approved by the institutional ethical review board of Haiyang People’s Hospital, Haiyang, Shandong, China. All the experiments were conducted as per Helsinki’s Declaration for human volunteers. All subjects gave informed, signed consent to participate in the study by themselves (reference no. 0067/IRB-LC/2017).

**Statistical analysis**

The SPSS 21.0 statistical software was used as statistical tool. The student t-test was used for comparison of the data of two groups. P-value (5%; P < 0.05) was considered statistically significant.

**Results**

**The comparison of microcirculation indicators**

Before the treatment initiation, there was no significant difference (P > 0.05) in the levels of NO, ET-1, TXB2, 6-Keto-PGF-1α, and SOD between the two groups. After 8 weeks of treatment, the levels of NO, 6-Keto-PGF-1α, and SOD in both groups were significantly higher than pre-treatment, while the levels of ET-1 and TXB2 were lower than pre-treatment.

After applying the t-test, the levels of NO, 6-Keto-PGF-1α, and SOD in the study group were significantly higher than the control group, while the levels of ET-1 and TXB2 in the study group were lower (P < 0.05) than the control group as shown in Table 1.
European Journal of Inflammation

The comparison of blood lipid detection levels

Prior to the treatment, there was no statistical significant difference ($P > 0.05$) in the levels of TC, TG, LDL, and HDL of the two groups. After 8 weeks of treatment, the levels of TC, TG, and LDL in the study group were significantly lower, while the levels of HDL were significantly higher than pre-treatment comparing to the control group. After 8 weeks post-treatment in the control group, the TC level was lower than pre-treatment ($P < 0.05$), while the HDL level was higher than pre-treatment ($P < 0.05$) as shown in Table 2.

The comparison of inflammatory factors and characteristic markers

Before the treatment, there was no significant difference ($P > 0.05$) in CRP, TNF-α, and IL-6 levels between the two groups. After 8 weeks of treatment, the levels of CRP, TNF-α, and IL-6 were significantly lower in the study group than the control group ($P < 0.05$). For control group, there was no significant difference in the above indicators on pre- and post-treatment ($P > 0.05$). There was no significant difference in the levels of VEGF, CysC, and Hcy between the two groups pre-treatment ($P > 0.05$). Post-treatment, the levels of VEGF, CysC, and Hcy in two groups were lower than those pre-treatment ($P < 0.05$). The above indicators in the study group were lower than those in the control group ($P < 0.05$). The comparison is shown in Table 3.

Discussion

Diabetes mellitus could cause organ damage and various complications, and may be a risk factor for aggravating the pathological changes in kidneys, liver, and other organs. Therefore, diabetic patients who have impaired renal and liver function must control blood sugar as soon as they are diagnosed.6,7

Mere lifestyle intervention cannot control blood sugar effectively. Most patients need combination therapy to maintain good blood glucose control. Therefore, once diabetes is diagnosed, drug control should be applied at the same time of lifestyle intervention.8,9

The statins are 3-hydroxy-3-methylglutaryl coenzyme (AMG-CoA) reductase inhibitors, Table 1. Comparison of microcirculation indicators in two groups ($\bar{x} \pm s$).

| Indicators | Control group (n = 85) | Study group (n = 85) |
|------------|-----------------------|---------------------|
|            | Pre-treatment | Post-treatment | Pre-treatment | Post-treatment |
| NO (jxmol/L) | 43.7 ± 4.6 | 55.4 ± 5.3* | 43.9 ± 5.1 | 66.6 ± 7.8*** |
| ET-1 (pg/L) | 95.6 ± 7.3 | 87.5 ± 6.2* | 95.1 ± 6.4 | 75.3 ± 3.5*** |
| TXB2 (ng/L) | 83.4 ± 6.5 | 66.3 ± 5.8* | 83.7 ± 7.2 | 80.8 ± 2.7*** |
| 6-Keto-PGF-1α (ng/L) | 53.2 ± 7.3 | 61.4 ± 6.2* | 53.4 ± 8.1 | 75.9 ± 6.4*** |
| SOD (U/mL) | 73.1 ± 6.6 | 82.3 ± 7.5* | 73.4 ± 6.2 | 94.5 ± 7.3*** |

NO: nitric oxide; ET-1: endothelin-1; TXB2: thromboxane-2; 6-Keto-PGF-1α: 6-ketone-prostaglandin F-1α; SOD: superoxide dismutase.

* $P < 0.05$, compared to pre-treatment.

** $P < 0.05$, compared to control group.

Table 2. The comparison of blood lipid detection levels in two groups (mmol/L, $\bar{x} \pm s$) (n = 85).

| Group | TC | TG | LDL | HDL |
|-------|----|----|-----|-----|
|       | Pre-treatment | Post-treatment | Pre-treatment | Post-treatment | Pre-treatment | Post-treatment | Pre-treatment | Post-treatment |
| Control | 9.7 ± 2.1 | 7.6 ± 1.3 | 3.4 ± 0.8 | 3.0 ± 0.8 | 4.6 ± 0.6 | 4.1 ± 0.4 | 0.7 ± 0.3 | 0.9 ± 0.4 |
| Study | 9.6 ± 2.2 | 5.1 ± 1.2 | 3.4 ± 0.6 | 1.7 ± 0.5 | 4.5 ± 0.7 | 2.6 ± 0.3 | 0.7 ± 0.4 | 1.6 ± 0.6 |
| t | 0.416 | 5.507 | 0.502 | 6.044 | 0.738 | 6.726 | 0.594 | 6.013 |
| P | >0.05 | <0.05 | >0.05 | <0.05 | >0.05 | <0.05 | >0.05 | <0.05 |

TC: total cholesterol; TG: triacylglycerol; LDL: low-density lipoprotein; HDL: high-density lipoprotein.
which are widely used in the treatment of abnormal lipid metabolism, which could effectively regulate the levels of TC and low-density lipoprotein cholesterol (LDL-C), and inhibit the action of the liver synthesis of apolipoprotein B-100, in order to achieve the purpose of reducing the synthesis and secretion of TG-rich lipoproteins. Atorvastatin is one of the important statins and is a hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor; by inhibiting the synthesis of liver HMG-CoA reductase and cholesterol, it lowers plasma cholesterol and lipoprotein levels, enhances the uptake and catabolism of LDL, and effectively reduces blood lipids. It has been suggested that atorvastatin can reduce the level of proteinuria and protect the renal function in the early stage of DN. The patients with DN often present renal microcirculation disorders, lipid metabolism dysfunctions, and inflammatory reactions. This study found that after 8 weeks of treatment of atorvastatin, the levels of NO, 6-Keto-PGF-1α, and SOD were significantly higher than pre-treatment, while the levels of ET-1, and TXB2 were lower. Similarly, after 8 weeks of treatment of the study group, the levels of TC, TG, and LDL were significantly lower, while level of HDL was significantly higher, which indicate a positive role of statin. Regulating protein kinase C (PKC) activation and ceramide biosynthesis could be a protective measure in the therapeutic potential of DN. Lipid-lowering drugs also upregulate anti-fibrotic microRNAs, which could hint at the effects of lipid-lowering drugs in DN. In post-treatment of study group, the levels of CRP, TNF-α, and IL-6 were significantly lower than pre-treatment and in the control group. There was no significant difference for the above indicators in the control group of the pre- and post-treatment. The VEGF is one of the important pro-angiogenic factors and is closely related to DN. And, the CysC is a reflection of glomerular filtration function and sensitive marker for early renal impairment. Hcy is associated with the severity of diabetic microangiopathy. In conclusion, on the basis of conventional treatment, the addition of atorvastatin treatment for patients with DN could effectively improve the renal microcirculation disorders, effectively correct the lipid metabolism dysfunction, relieve the level of inflammatory response, and reduce the renal impairment to achieve the purpose of protect the kidneys function.

### Table 3. The comparison of serum inflammatory factor levels and characteristic markers by two groups (mmol/L, ± s).

| Group     | CRP    | TNF-α  | IL-6   | VEGF (pg/mL) | CysC (mg/L) | Hcy (μmol/L) |
|-----------|--------|--------|--------|--------------|-------------|--------------|
|           | Control| 106.8 ± 15.2 | 96.6 ± 12.7 | 254.8 ± 13.2 | 231.3 ± 19.7 | 6.7 ± 1.5 |
|           | Study  | 107.2 ± 15.7 | 77.5 ± 13.2 | 253.3 ± 22.4 | 218.7 ± 15.4 | 6.3 ± 1.3 |
|           | L      | 0.126 | 0.0139 | 0.0207 | 0.0226 | 0.0196 |
|           | p      | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |
|           | t      | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |

CRP: C-reactive protein; TNF-α: tumor necrosis factor alpha; IL-6: interleukin-6; VEGF: vascular endothelial growth factor; CysC: cystatin C; Hcy: homocysteine.
Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

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