Qizhi decoction prevent diabetic nephropathy through reducing inflammation and protection of endothelial cells
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ABSTRACT

Background: Low grade inflammation and endothelial cells dysfunction are the basic pathological change of diabetic nephropathy (DN). Two or three kinds of Chinese herbs combined together organically can resolve these problem at the same time. It costs less money and have less side effects than chemical medicine does.

Materials and Methods: One hundred and fifty five patients diagnosed with DN were randomly divided into two groups. The treatment group was treated with Chinese medicine, the Qizhi decoction, in combination with symptomatic treatment with Western medicine, whereas the control group received only Western medicine. The serum inflammatory parameters C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), monocyte chemo-attractant protein-1(MCP-1), and the endothelial cells dysfunction parameters endothelin (ET1), nitric oxide (NO), Intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1(VCAM-1) were evaluated.

Results: Glycosylated hemoglobin and plasma glucose levels were lower in the treatment group, albeit without significance. CRP, TNF-α, MCP-1, ET1, ICAM-1, VCAM-1 declined significantly in the treatment groups after treatment. NO increased in the treatment groups after treatment. The urine albumin level and urine protein/creatinine ratio significantly decreased in groups after treatment. Conversely, no obvious changes were observed in the control group.

Conclusion: The Qizhi decoction can significantly reduce chronic inflammation and protection endothelial cells in patients with diabetic nephropathy.

Keywords: Chinese medicine; inflammation; endothelial cell; diabetic nephropathy
INTRODUCTION

Diabetes represents one of the major health threats facing humans. The World Organization (WHO) has called diabetes the epidemic of the 21st century. WHO estimated that more than 180 million people worldwide suffer from diabetes today and predicts that the number will double before 2030. Diabetic complications increase in severity and frequency as diabetes progresses. Almost 80% of patients with diabetes die from diabetic complications opposed to the poor control of plasma glucose levels. Approximately 30–35% of patients with diabetes develop diabetic nephropathy (DN), the most frequent cause of end-stage renal disease (ESRD). 1,2 Many patients develop kidney damage despite all pharmacologic therapies available for DN treatment. Even today there is no successful chemical therapy for diabetic nephropathy resulting from blood vessels malfunction. In many cases the only option is to wait for dialysis. We need of complete understanding of molecular, metabolic and environmental factors that lead to DN and their interaction between them. 3 Human endothelial cells line the interior surface of all blood vessels and perform an array of functions critical to vascular health. Endothelium is not an inert, single-cell lining covering the internal surface of blood vessels, but in fact plays a crucial role in regulating vascular tone and structure. Importantly, a healthy endothelium inhibits platelet and leukocyte adhesion to the vascular surface and maintains a balance of profi brinolytic and prothrombotic activity. It has received the status of an organ, albeit a widely spread one, which is responsible for the regulation of the hemodynamics, angiogenic vascular remodeling, and metabolic, synthetic, inflammatory, antithrombogenic, and prothrombogenic processes. Endothelial cells (ECs) are capable of secreting a variety of biological mediators to maintain vascular hemostasis and prevent thrombotic complications. Under normal circumstance, endothelial cells are able to metabolize circulating glucose, providing the energy that the body needs to function. 4 Endothelial cell dysfunction (ECD) means dysregulation of endothelial cell functions, including impairment of the barrier functions of endothelial cells, vasodilation, disturbances in proliferative capacities, migratory as well as tube formation properties, angiogenic properties, attenuation of synthetic function, and deterrence of white blood cells from adhesion and diapedesis. 5 It implies diminished production or availability of nitric oxide (NO) and the imbalance of endothelium-derived relaxing and contracting factors, such as endothelin-1 (ET-1), angiotensin, and oxidants. NO is the key endothelium-derived relaxing factor that plays a pivotal role in the regulation of vascular tone and vasmotor function. In addition to its vasodilatory effect, NO also protects against vascular injury, inflammation, and thrombosis. 6,7 The trigger for endothelial cell dysfunction in diabetes is hyperglycemia, but the maintenance phase is tightly linked to the accumulation of AGEs. Angiotensin II, opposes NO action, can elicit the production of reactive oxygen species, increase the expression of the proinflammatory cytokines interleukin (IL)-6 and monocyte chemoattractant protein-1 (MCP-1), and upregulate vascular cell adhesion molecule-1 (VCAM-1) on ECs. When ECs undergo inflammatory activation, the increased expression of selectins, VCAM-1, and intercellular adhesion molecule-1 (ICAM-1) promotes the adherence of monocytes. Once adherent, the monocytes transmigrate into the inner layer of the arterial wall, passing between the ECs. Adhesion molecule expression is induced by proinflammatory cytokines such as IL-1 and tumor necrosis factor-α (TNF-α). Elevated C-reactive protein (CRP) levels can promote endothelial dysfunction by quenching the production of NO and diminishing its bioactivity. CRP potently down regulates endothelial NO synthase (eNOS) transcription in ECs and destabilizes eNOS mRNA, result in decreases in both basal and stimulated NO release. At the same time, CRP can stimulate ET-1 and IL-6 release, upregulate adhesion molecules, and stimulate MCP-1 while facilitating macrophage LDL uptake. Impaired endothelium dependent vasodilation is a prominent feature in patients with moderate to advanced renal impairment treated by haemodialysis or peritoneal dialysis. 8,9 In addition, chronic inflammation is involved in pathogenesis of DM and as a result DN. Some studies have demonstrated that cytokines, chemokines, growth factors, adhesion molecules, nuclear factors as well as immune cells as monocytes, lymphocytes and macrophages are all involved in DM pathogenesis and of course play an important role in DM complications. 10 MCP-1 can promote transformation of monocytes in macrophages. Macrophage produce diverse cytokines as IL-6 and TNF-α. Patients with urinary albumin excretion presented higher circulating levels of MCP-1 than patients without this alteration. These findings suggest that MCP-1 plays an important role in pathogenesis of DN as the protein produced not only in vascular wall, but also in tubular epithelial cells. Inflammatory cytokines as TNF-α, MCP-1 promote renal fibrosis, leading to renal failure in patients with DN. 11,12 The evidence suggest that TNF-α, MCP-1, adhesion molecules and Endothelial cell dysfunction have a prominent role in the development of DN, and all these mediators may be considered therapeutic targets for the prevention and treatment of DN. Blocking the
activity of inflammatory cytokines and protection of ECs may be an important strategy for preventing DN. In this study, we assessed whether the Chinese medicine Qizhi decoction can prevent such complications as a treatment for DN.

**Methods**

**Patients:**
This is a prospective study. Patients were targeted for enrollment among stage II to stage IV diabetic nephropathy who regularly attended the Guangdong General Hospital for outpatient and inpatient departments treatment. We enrolled 155 patients. The treatment group consisted of 78 patients (40 males, 38 females; age, 48–69 years; mean age, 56.81 ± 6.37 years). The duration of disease in the treatment group ranged from 5 to 15 years (mean, 10.15 ± 1.32 years). The control group consisted of 75 patients (36 males, 37 females; age, 47–70 years; mean age, 57.12 ± 1.01 years). The duration of disease in the control group ranged from 6 to 16 years (mean, 9.41 ± 2.53 years). The two groups were similar in terms of patient gender, duration of disease, and patient conditions (P > 0.05).

**Diagnostic criteria:**
Western diagnostic criteria (refer to 1999 WHO/ADA criteria) patients with a history of diabetes and persistent microalbuninuria (urinary albumin excretion rate [UAER] = 20–200 µg/min or 30–300 mg/day) were diagnosed with early DN, whereas those with proteinuria or large amounts of urinary protein and nephrotic syndrome were diagnosed with clinical DN.

**Traditional chinese medicine (TCM) standards** (refer to criteria of “Guideline for TCM Diabetes Prevention and Treatment” of the China Association of Chinese Medicine) The symptoms of Qi-yin asthenia include proteinuria, lassitude, shortness of breath, dizziness, dreaminess, frequent urination, palp and planter fever, palpitations, a thin and red (or pink) tongue, and a weak pulse. The signs of liver kidney yin insufficiency include proteinuria, vertigo, tinnitus, burning sensation of the five centers, soreness of the waist and knees, dry eyes, a red tongue, less moss, and a rapid pulse. The symptoms of Qi-blood asthenia include proteinuria, lassitude, shortness of breath, a pale or sallow complexion, dizziness, colorless lips and nails, palpitations, insomnia, soreness of the waist and knees, a pale tongue, and a weak pulse. The signs of spleen-kidney yang deficiency include proteinuria, mental fatigue, chills, swollen limbs (especially the lower limbs), a pale face, long or deficient urine, an increase in nocturia or diarrhea before dawn, a pale tongue with a fat body, and a slow and weak pulse.

**Inclusion criteria:** Patients who met the Chinese and Western diagnostic criteria were selected for the study.

**Exclusion criteria:** Patients who do not meet the diagnostic criteria; those with kidney disease caused by other diseases; pregnant or lactating women; children; patients with other diseases or complications (e.g., congestive heart failure, elevated serum transaminase levels, primary hypertension); those with other serious heart, brain, lung, liver and other primary organ diseases; those with diabetic ketoacidosis or urinary tract infection within the previous month; those with severe infections and other complications; those with malignant hypertension or myocardial infarction within the past 6 months, those with a history of cerebrovascular accident; and those with a recent history of nephrotoxic drug use were excluded.

**Treatment:** We conducted a single-center, open-label, randomized clinical trail to evaluate the effect of Qizhi decoction on DN. The patients were numbered and grouped according to the staging criteria for DN. Then, the patients were assigned a zheng differentiation-classification according to the TCM diagnostic criteria. All patients were randomly assigned to the treatment or control group using a random number table. Both groups completed 3 months of diet control and exercise therapy. The patients in the control group were routinely given gliclazide (approval number: national drug approval number H20044694) or insulin to reduce blood glucose levels. Patients with hyperlipidemia were given simvastatin (approval number: national drug approval number H2001677) and symptomatic treatment simultaneously. Patients in the control group were given irbesartan (approval number: H20080074) to reduce blood pressure. The treatment group was administered a daily dose of the Qizhi decoction (Astragalus, Leeches,Cornus). The medicine was decocted with water twice and taken twice after mixing. Blood and urine specimens were collected before and after treatment. Participants were not allowed to take any other TCM, Chinese patent medicine to stimulate the liver or kidneys or to activate blood circulation, or other drugs that could interfere with the agents used in the study.

The study protocol was approved by the institutional review board at Guangdong General Hospital and conducted in accordance with the Declaration of Helsinki and its amendments. After a full explanation of the study, all patients gave written informed consent.

**Measurements:** Before starting the study, all selected patients underwent an initial screening assessment that included a medical history and physical examination. We evaluated patients at the start of the study, then again after the 3rd month of treatment. Parameters were as follows: Routine blood sampling to assess liver and kidney function as well as glycosylated hemoglobin, fasting plasma glucose, and 2-h postprandial glucose levels. Urine albumin (UA) levels...
and the urine protein/creatinine ratio (U/C) were evaluated by radioimmunoassay. Plasma ET1, NO levels were determined by radioimmunoassay according to the instruction of the kits. Blood samples (7 ml) from patients with DN were withdrawn, while the subject or patient was in a sitting position, from the antecubital vein into K2-EDTA tubes placed on ice. Plasma was immediately separated and stored at -40°C until assayed. Plasma ET1 was measured by RIA. In short, a 2 ml aliquot of plasma was acidified with trifluoroacetic acid (TFA) and applied to a Spe-C8 cartridge, which had been prewashed sequentially with methanol, distilled water, and 0.09% TFA. The materials adsorbed to the cartridge were eluted with 60% acetonitrile/0.09% TFA. The materials adsorbed to the cartridge were eluted with 60% acetonitrile/0.09% TFA. The minimum detectable quantity of ET-1 in RIA was 0.5 pg/tube, and the 50% intercept was 15 pg/tube. The antibody mainly recognizes the C-terminal Trp21 residue of ET-1; it cross-reacted fully with ET-1, ET-2, and ET-3, although it did not show any cross-reactivities with big porcine ET-1 (1-39), big human ET-1 (1-38), or C-terminal fragment (22-39) of big porcine ET-1. Plasma concentrations of NO were determined by RIA as described previously. Plasma C-reactive protein (CRP), TNF-α, and MCP-1, VCAM-1, ICAM-1 levels were determined by ELISA (enzyme-linked immunosorbent assay) according to the instruction of the kits.

**Statistical analysis:** Statistical analysis was conducted using Statistical Package for Social Sciences software version 19.0. The data are presented as the mean ± S.E. Student’s t-test was performed for comparisons between groups, and the χ² test was used for numerical data. For all statistical analysis, P < 0.05 was considered statistical significant.

**Results**

**Comparison of the overall efficacy of the two treatments:**

155 patients were enrolled in the study and of these 145 completed the study. The reason for premature withdrawal was lost-to-follow-up. The treatment group exhibited greater improvements in clinical symptoms and larger reductions in urinary protein levels (P < 0.01). The combination therapy appeared to be more effective than Western medicine alone, as shown in Table 1.

<table>
<thead>
<tr>
<th>Clinical control</th>
<th>Markedly effective</th>
<th>Effective</th>
<th>Ineffective</th>
</tr>
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<tbody>
<tr>
<td>Treatment group</td>
<td>25 (33.3%)</td>
<td>30 (40%)</td>
<td>17 (22.7%)</td>
</tr>
<tr>
<td>Control group</td>
<td>18 (24.29%)</td>
<td>23 (32.86%)</td>
<td>12 (17.14%)</td>
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</table>

Table 1 Comparison of the overall efficacy of the two treatments (n [%])

☆, P < 0.01 compared with the control group.

**Changes in urine albumin levels and the urine protein/creatinine ratio:** As indices of early DN, urine albumin levels decreased obviously in treatment group after treatment. The urine protein/creatinine ratio decreased noticeably in treatment group after treatment. Compared with the control group, greater decreases were observed in the treatment group, as shown in table 2.

<table>
<thead>
<tr>
<th></th>
<th>Treatment group</th>
<th>Control group</th>
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</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>UA (mg/L)</td>
<td>2564.6±45.8</td>
<td>170.8±24.8</td>
</tr>
<tr>
<td>U/C (mg/mmolCr)</td>
<td>2.35±0.43</td>
<td>1.25±0.36</td>
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</table>

Table 2 Changes in UA levels and the urine U/C ratio in patients with DN

△, P < 0.01 compared with before treatment; ☆, P < 0.01 compared with the control group.

**Changes in CRP and TNF-α levels in the two groups:**

Compared with the control group, CRP, TNF-α and MCP-1 were substantially improved in the treatment groups after treatment. But the change in control group was mean less. See Table 3.

<table>
<thead>
<tr>
<th></th>
<th>Treatment group</th>
<th>Control group</th>
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</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>8.86±1.31</td>
<td>412±218</td>
</tr>
<tr>
<td>TNF-α (ng/L)</td>
<td>22.36±2.13</td>
<td>1445±356</td>
</tr>
<tr>
<td>MCP-1 (1ng/ml)</td>
<td>14.32±4.35</td>
<td>5.56±2.42</td>
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</tbody>
</table>

Table 3 Changes of CRP, TNF-α and MCP-1 levels in the two groups

△, P < 0.01 compared with before treatment; ☆, P < 0.01 compared with the control group.

**Changes in ICAM-1, VCAM-1 levels in the two groups:**

The results indicated that ICAM-1, VCAM-1 declined noticeably after treatment in the treatment group, whereas less obvious change was observed in the control group. See Table 4.

<table>
<thead>
<tr>
<th></th>
<th>Treatment group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td>2826±1431</td>
<td>1612±12.18</td>
</tr>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td>3543±1913</td>
<td>17445±13.56</td>
</tr>
</tbody>
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Table 4 Changes in ICAM-1 and VCAM-1 levels in the two groups (△)

△, P < 0.01 compared with before treatment; ☆, P < 0.01 compared with the control group.
Changes in ET1, NO in the two groups:
The results indicated that ET1 declined noticeably after treatment in the treatment group, whereas less obvious change was observed in the control group. NO increased noticeably after treatment in the treatment group. But less change in control group is seen. See Table 5.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Control group</th>
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<tbody>
<tr>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>ET (pg/ml)</td>
<td>188.96±5.31</td>
</tr>
<tr>
<td>NO (μmol/l)</td>
<td>94.36±6.13</td>
</tr>
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</table>

Table 5 Changes in ET1 and NO levels in the two groups (±s)

Discussion
Diabetic nephropathy (DN) is a serious microvascular complication of diabetes, is the leading cause of end-stage renal disease and affects an estimated 150 million people worldwide. Despite optimal treatment, including glycemic control and antihypertensive therapy, the disease progresses. Multiple factors contribute to the occurrence and development of DN. Chronic inflammation, which can promote glomerular and interstitial fibrosis, is the primary cause of renal failure in patients with DN. However, because of multiple pathways that joint inflammation with diabetic complications, it looks unlikely that one single molecule be sufficient for the development of DN. Blocking the activity of inflammatory cytokines or the principal mediators may is useful in the prevention of this complication. TNF-α is primarily produced by monocytes (macrophages) and T cells. Interstitial, glomerular, and tubular cells can also synthesize and secrete TNF-α. TNF-α may alter the barrier function of the glomerular capillary wall and increase vascular permeability by activating Fas signaling pathway and promoting the production of local reactive oxygen radicals, leading to the production of urinary protein.

Urinary albumin excretion is apparently related with TNF-α mRNA levels in the renal cortex and the TNF-α concentration in urine. Urinary albumin excretion is the clinical sign of early DN and the most important factor during the progression of ESRD. Thus, an increase in TNF-α levels is a significant marker of DN. TNF-α is one of the key cytokines that promote endothelial cell injury by inhibiting NO synthase, resulting in reduced NO production and increased NO degradation. TNF-α can attenuate the vasodilatory effect of NO. Thus, the renal vessels are constricted, resulting in hemodynamic balance disorders and changes in renal blood flow and glomerular GFR. Renal tubular epithelial cells exposed to TNF-α can synthesize and secrete lymphocytes, neutrophils, and chemokines. Intercellular adhesion molecule-1 (ICAM-1) expression on the cell surface is also increased significantly in response to TNF-α. TNF-α can induce the proliferation of fibroblasts and endothelial procoagulant activity and stimulate prostaglandin synthesis. Therefore, increases in TNF-α levels can alter glomerular microcirculation, reduce renal blood flow and GFR, and promote renal failure, findings that are closely related to the theory of blood stasis in TCM. TNF-α is an important pro-apoptotic factor that promotes connective tissue growth factor (CTGF) expression, resulting in the induction of apoptosis in glomerular and interstitial cells. In addition, TNF-α is cytotoxic to glomerular, mesangial, and epithelial cells, resulting in direct harm to the kidneys. TNF-α synthesis and release also accelerates renal tubular sodium transport, causing sodium retention and resulting in kidney hypertrophy, which is the pathological change of early DN. Clinical studies revealed that TNF-α levels in the serum and urine and urinary albumin excretion have obvious and independent correlations in patients at various stages of DN. Urinary TNF-α levels are increased in patients with diabetes who exhibit increases in urinary albumin excretion. During the progression of DN, urinary TNF-α excretion also increased significantly. Anti-TNF-α antibodies, mycophenolate, and statins can reduce urinary protein levels and improve renal function. Thus, TNF-α plays an important role in the occurrence and development of DN, and it may be an important target in the treatment of this illness.

MCP-1 is a major pro-inflammatory cytokine. Mesangial and tubular epithelial cells can secrete MCP-1. MCP-1 participates in the occurrence and development of DN through the accumulation and activation of monocytes (macrophages). High levels of glucose, advanced glycosylation end products, angiotensin II, and mechanical stretch (glomerular hypertension) can stimulate the renal parenchyma and mesangial cells to secrete MCP-1. MCP-1 can stimulate mesangial cells and other cells to secrete large amounts of inflammatory cytokines and pro-inflammatory cytokines in a paracrine manner. MCP-1 can promote the release of adhesion molecules (vascular cell adhesion molecule-1; ICAM-1) and increase their activity, thus contributing to inflammatory cell adhesion to vascular endothelial cells, exudation, and aggregation and a subsequent intensification of local inflammation. MCP-1-activated monocytes (macrophages) secrete large amounts of growth factors, leading to fibroblast proliferation and differentiation. MCP-1 can directly induce endothelial cells and epithelial cell transdifferentiation and induce the synthesis of fibronectin, collagen fiber protein IV, and extracellular matrix, ultimately aggravating fibrosis and promoting glomerular and interstitial fibrosis.
The endothelin (ET) system is strongly involved in the pathology of DN and contributes to vasoconstriction, inflammation, and proliferation. ET antagonists are promising drugs that potently slow down disease progression in animal models. However, the available ET antagonists may also inhibit tubular endothelin receptors subtype B, which promote sodium and water excretion. Fluid retention, oedema and, in higher stages of chronic kidney disease, heart failure limit the use of ET antagonists. Endogenous NO is produced through the conversion of the amino acid, L-arginine to L-citrulline by NO-synthase (NOS). NO produced by NOS type III in the endothelium diffuses to the vascular smooth muscle (VSM) where it activates the enzyme guanylate cyclase. The concomitant increase in cyclic GMP then induces relaxation of the VSM.

Microalbuminuria is usually defined as a urinary albumin excretion rate of 30–300 mg in a 24 h urine collection, or as a urinary albumin excretion rate of 20–200 mg/min in a timed overnight urine collection. It is an independent risk factor for the development of cardiovascular disease and a predictor of cardiovascular mortality in the diabetic population. It is related to endothelial dysfunction, increased oxidative stress, and diabetic glomerulosclerosis in diabetic persons.

Microalbuminuria levels and the urine protein/creatinine ratio are common indices for the diagnosis of DN. Our study showed that the index in patients with DN usually increased at different degrees and could relieve after treatment. Additionally, the effect of combined treatment with TCM and Western medicine on these variables was greater than that of Western medicine alone.

Our previous studies revealed that Chinese medicine such as cortex moutan, which can activate blood flow, and other TCMs, which can stimulate the kidneys and dredge collaterals, can reduce the expression of CTGF, CRP, and other inflammatory cytokines in patients with DN. This study demonstrated that the Qizhi decoction can both improve the clinical symptoms of DN and reduce CRP, TNF-α, and MCP-1, VCAM-1, ICAM-1, ET1, levels, reduce urine albumin (UA) levels and the urine protein/creatinine ratio, increase the level of NO. The results indicating that the decoction may alleviate the inflammatory response and protect the epithelial cell in patients with DN and effectively prevent DN effectively.

Acknowledgment

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