Study on PINK1 Expression and Its Clinical Value in Diabetic Nephropathy

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Introduction. To explore PTEN-induced putative kinase 1 (PINK1) expression and its clinical value in diabetic nephropathy.
Methods. Ninety patients with diabetic nephropathy were recruited and divided into metformin hydrochloride monotherapy group, telmisartan monotherapy group and combination therapy (metformin and telmisartan) group. Renal function indices and PINK1 expression, inflammatory factors, reactive oxygen species (ROS), mitochondrial membrane potential (MMP) levels were detected. The correlation between PINK1 and inflammatory factors, renal function indicators including estimated glomerular filtration rate (eGFR), urinary albumin-to-creatinine ratio (UACR) and serum creatinine (SCr)] were analyzed by Pearson correlation.
Results. Following treatments, the combination therapy group exhibited increased PINK1 expression levels and decreased ROS levels compared to the groups receiving metformin hydrochloride or telmisartan monotherapy. The combination therapy group showed significant improvements in renal function indices and inflammatory markers. Additionally, the MMP ratio in the combination therapy group was higher compared to the two monotherapy groups. Furthermore, PINK1 was negatively correlated with UACR, SCr, tumor necrosis factor (TNF-α) and interleukin-6 (IL-6), while positively correlated with eGFR and interleukin-2 (IL-2).
Conclusion. PINK1 exhibits low expression levels in patients with diabetic nephropathy and its expression is strongly associated with the inhibition of disease progression, thereby offering significant clinical diagnostic value. Additionally, it may serve as a potential biological marker for clinical diagnosis and treatment of diabetic nephropathy.

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Original Paper

INTRODUCTION

Diabetic nephropathy is a progressive decrease which presents with albuminuria and reduced glomerular filtration rate (GFR).[1] Diabetic nephropathy, a significant consequence of diabetes mellitus,[2] is becoming more prevalent in China and has emerged as the second cause of end-stage kidney disease after various glomerulonephritis, such as chronic glomerulonephritis.[3] Due to complicated metabolic disorders, diabetic nephropathy is often more difficult to treat than other kidney diseases once it progresses into end-stage kidney disease, so timely prevention and treatment are essential for delaying diabetic nephropathy.[4]

The development of diabetic nephropathy is closely linked to mitochondrial function.[5] Increasing evidence has revealed that mitochondrial dysfunction contributes to the progression of

diabetic nephropathy.[6] Since kidneys are the second highest oxygen consuming organ in the body and are significantly sensitive to mitochondrial dysfunction,[7] and high blood glucose induces mitochondrial cholesterol accumulation and associated injury in DN; high blood glucose can directly damage renal tubular cells, leading to widespread metabolic and cellular dysfunction.[8]

Reactive oxygen species (ROS) belong to a general term for oxygen-containing free radicals linked to oxygen metabolism in living organisms and peroxides that easily form free radicals.[9] ROS overproduction activates many signaling pathways and induces the release of many cytokines, pro-inflammatory markers as well as growth factors, which indirectly leads to tissue and cell damage, and plays a critical role in the progression of diabetic nephropathy.10

PTEN-induced putative kinase 1 (PINK1) belongs to protein kinase family, and is expressed in cells throughout the body, particularly in energy-intensive organs such as heart, muscles, and brain.[11] Within the cells, it is mainly presented in the inner membrane of the mitochondria.[12] It has been hypothesized that during periods of cellular stress, such as when the cell has unusual high energy requirements, it functions as a protector of mitochondria.[13] PINK1 has been reported to be involved in diabetic nephropathy progression.[14] However, clinical value of PINK1 in diabetic nephropathy need to be further investigated. Therefore, it is of great significance to explore PINK1 expression as well as its potential clinical value in diabetic nephropathy.

MATERIALS AND METHODS

General data

Based on the inclusion and exclusion criteria, 90 patients with diabetic nephropathy at different stages, admitted to Longquan City People's Hospital (Zhejiang, China) from January 2022 to December 2023, were selected according to inclusion and exclusion criteria and randomly divided into metformin hydrochloride monotherapy group, telmisartan monotherapy group and combination therapy (metformin hydrochloride plus telmisartan) group. Each group had 30 patients, and the course of treatment was three months. Inclusion criteria were patients: (1) with a clear history of type 2 diabetes mellitus; (2) aged between 25 and 70 years old; (3) with a diabetes course of more than five years; (4) with the diagnosis criteria of diabetic nephropathy stage 1 to 4.[15] Exclusion criteria were patients: (1) with serious diseases such as cerebrovascular accident and myocardial infarction in the past six months; (2) receiving an angiotensin-converting enzyme inhibitor (ACEI)/angiotensin-ii receptor blocker (ARB) or metformin formulation within 4 weeks; (3) with diabetic nephropathy stage 5; (4) with the presence of other primary or secondary kidney diseases; (5) with urinary tract infection, autoimmune disease, and malignant tumor. Our work was approved by the Medical Ethics Committee of Longquan City People's Hospital. All the study participants have signed informed consent.

Treatment methods

Metformin hydrochloride. The metformin hydrochloride was started with a 0.5 g twice daily or 0.85 g once daily with meals, and gradually increased by 0.5 g per week, or 0.85 g every 2 weeks, up to 2 g per day, taken in batches, according to the patient's condition. The maximum recommended dose for adults was 2550 mg daily. For patients who needed further control of blood glucose, the dose could be increased to 2550 mg daily (0.85 g each time, three times a day). When the daily dose was more than 2 grams, for better tolerance, the drug was best taken with three meals.

Telmisartan. For adults whose blood pressure could not be adequately controlled by telmisartan alone, telmisartan was given once a day with water, before or after meals. It was recommended that the two components in the compound preparation be dose-titrated separately before switching to the compound preparation. When appropriate, direct conversion of telmisartan monotherapy to a combination might also be considered. For patients whose blood pressure was not adequately controlled with telmisartan 80 mg, telmisartan hydrochlorothiazide tablets 80/12.5 mg could be given.

Medication for renal injury. Regular monitoring of renal function was recommended. For patients with mild to moderate liver function impairment, the dose did not exceed telmisartan hydrochlorothiazide tablet 40/12.5 mg once a day.

Observation indicators

(1) Peripheral blood of patients were collected

within 24 hrs after admission, 5 mL anticoagulant peripheral blood was centrifuged at room temperature for 10 min (3000 r/min), and the bottom-layer blood cells were collected and were centrifuged for 15 min (4℃, 12000 r/min), the upper layer clear liquid was removed, and the obtained blood cells were placed in aseptic centrifuge tubes at 500 μL per tube and stored at -80℃ for the detection of PINK1 mRNA expression. RT-qPCR was adopted to detect the relative mRNA expression level of PINK1 in peripheral blood cells. Total RNA was extracted from peripheral blood cells of each group according to the instructions of RNA kit. The cDNA was obtained by reverse transcription kit according to the instructions. The 2 μL cDNA was configured with a reaction system of 20 μL. The above cDNA was amplified by SYBR Green Master Mix, and the LightCycler 96 qPCR instrument was used for detection. The primer sequences were: PINK1-F: 5′-GCCTACATTGCCCCAGAACC-3′, PINK1-R: 5′-TGGAGGAACCTGCCGAGAT-3′. GAPDH-F: 5′-GGTGAAGCAGGCGTCGGA-3′, GAPDH-R: 5′-GGAGTGGGTGTCGCTGTTGA-3′. All the samples were set up with 3 double pores, and the relative mRNA expression of PINK1 was calculated by $2^{-\triangle\triangle Ct}$ method.

(2) Renal function indices of the three groups were compared. Urine samples were collected at 24 hrs, and special protein meter immunoscattering nephelometry was used as the detection method to detect urinary albumin (UALB) and the urinary albumin creatinine ratio (UACR) was calculated. Five mL of fasting venous blood was centrifuged at 3000 r/min for 10 min. Serum creatinine (SCr) was examined by an automatic biochemical analyzer produced by Zhongshan Xinrui Medical Equipment Technology Co., LTD. eGFR was examined by corresponding qPCR reagent kits.

(3) Inflammatory factor levels were compared between the three groups, and five mL of fasting venous blood was centrifuged at 3000 r/min for 10 min. The levels of tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6 and IL-2 were measured by enzyme-linked immunosorbent assay.

(4) ROS level was compared among the three groups. The procedure was as follows: Peripheral blood nucleated cells treated with erythrocyte lysate were incubated with chloro-methyl-dichlorofluorescein diacetate (CM-DCFDA, 10 mM) (Molecular Probes, USA). Samples were then centrifuged at 1000 × g for three min, and the pellets were resuspended in 500 μL phosphate-buffered saline (PBS), followed by analysis using flow cytometry (BD Biosciences, CA).

(5) Mitochondrial membrane potential (MMP) was compared among the three groups. MMP was assayed by using JC-1 (Beyotime, China). Briefly, peripheral blood nucleated cells treated with erythrocyte lysate were cultured in 6-well plates with 3×10^5 cells per well. After 24 hrs of growth, cells were stained with JC-1, and analyzed by flow cytometry (BD Biosciences, USA). The absorption values of green and red fluorescence were measured at 529 nm and 590 nm respectively, and the ratio of red fluorescence to green fluorescence was the MMP.

Statistical analysis

SPSS 22.0 statistical software was used to process the data. The measurement data were expressed as (x ± s), and measured by t test, one-way analysis of variance and SNK-q test. The correlation between PINK1 and inflammatory factors, renal function indicators was analyzed by Pearson correlation. $P < .05$ was considered as statistically significant.

RESULTS

PINK1 expression level among the three groups

As displayed in Figure 1, no difference was found in PINK1 expression level among the three groups prior to therapy ($P > .05$). After therapy, PINK1 expression level was elevated in all groups; however, it was higher in the combination therapy group than metformin hydrochloride monotherapy group and telmisartan monotherapy groups ($P < .05$).

Renal function indexes in the three groups

No difference in eGFR, UACR, SCr and UALB levels was found among the three groups prior to therapy ($P > .05$), as illustrated in Figure 2. After therapy, eGFR level was elevated, while UACR, SCr and UALB levels decreased in all groups. The improvements of the above indexes in the combination therapy group were more obvious than metformin hydrochloride monotherapy group and telmisartan monotherapy groups ($P < .05$).

Levels of inflammatory factors among the three groups

As shown in Figure 3, there was no difference

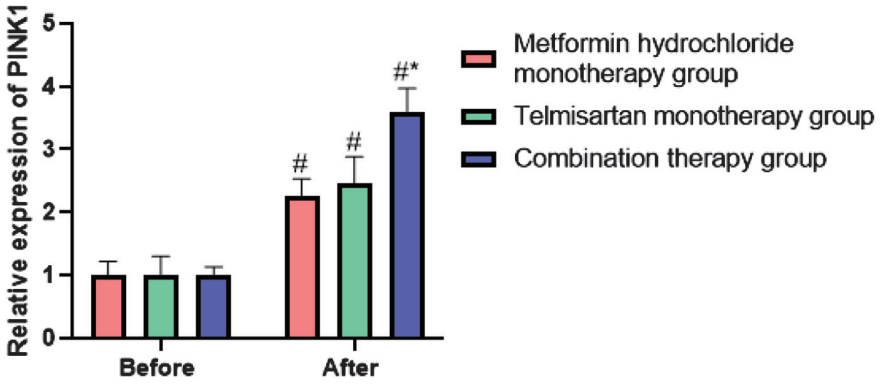

Figure 1. PINK1 expression level among the three groups. $P < .05$, compared with before therapy. $^{*}P < .05$, compared with metformin hydrochloride monotherapy group and telmisartan monotherapy group.

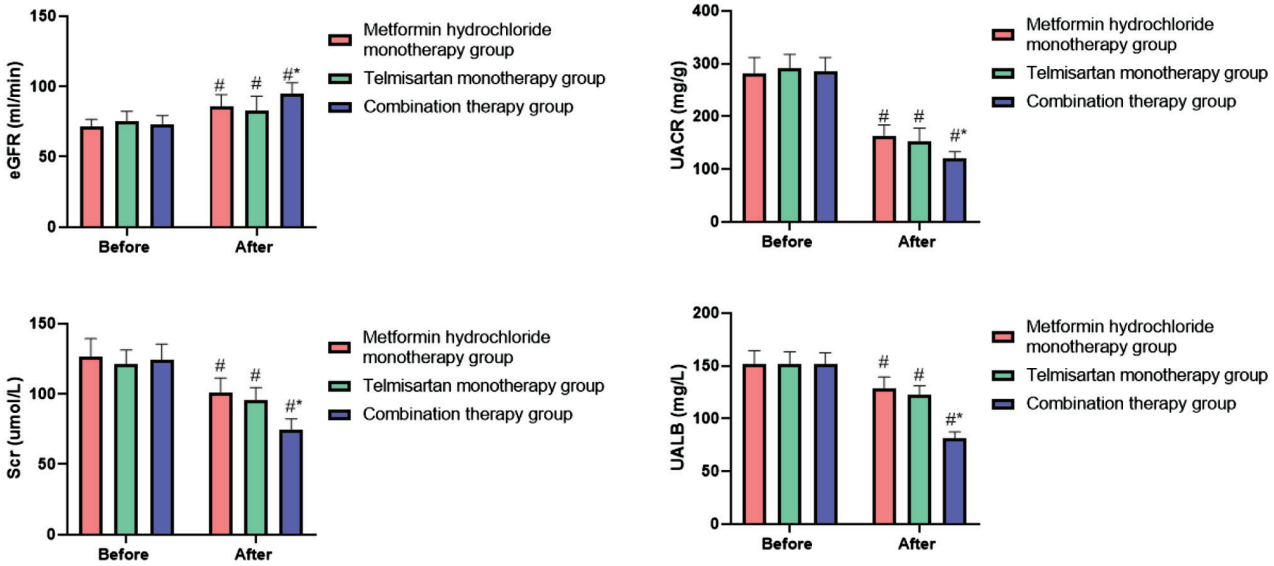

Figure 2. Renal function indices among the three in groups. $^{\#}P < .05$, compared with before therapy. $^{*}P < .05$, compared with metformin hydrochloride monotherapy group and telmisartan monotherapy group.

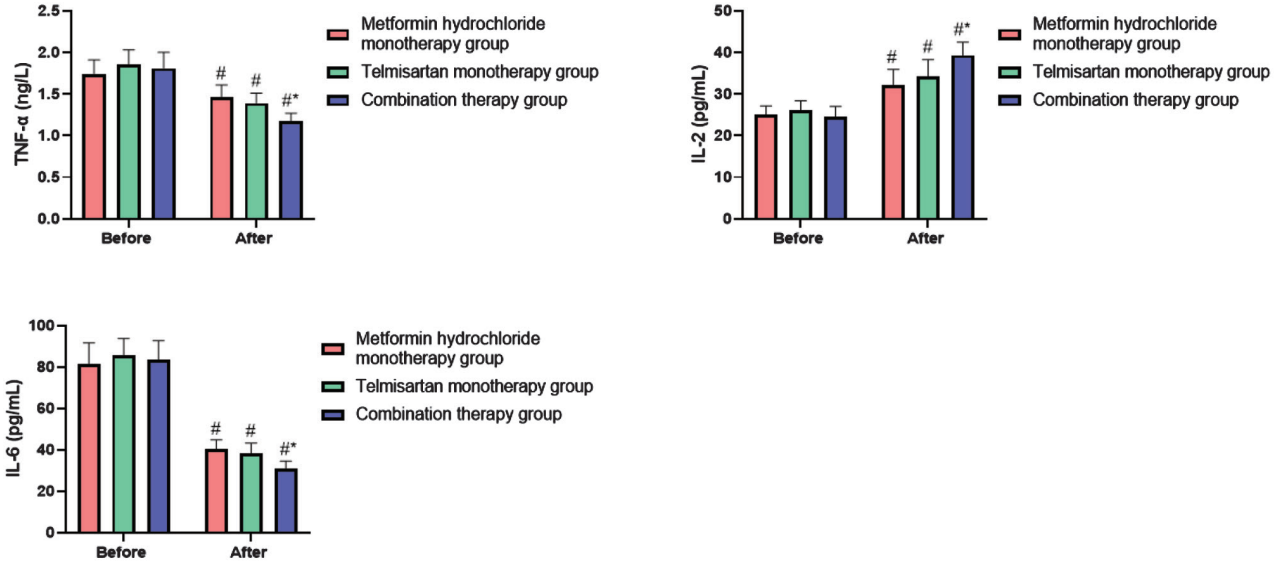

Figure 3. Levels of inflammatory factors among the three groups. $^{\#}P < .05$, compared with before therapy. $^{*}P < .05$, compared with metformin hydrochloride monotherapy group and telmisartan monotherapy group.

in TNF-α, IL-6 and IL-2 levels among the three groups before treatment ($P > .05$). After therapy, IL-2 level was elevated, while TNF-α and IL-6 levels decreased in all groups. The combination therapy group showed a greater improvement in the aforementioned indicators compared to the metformin hydrochloride monotherapy group and telmisartan monotherapy group. ($P < .05$).

ROS level among the three groups

Figure 4 shows no difference in ROS level among the three groups prior to therapy ($P > .05$). Following treatment, ROS level declined in all groups. Specifically, the combination therapy group exhibited less reduction in ROS than metformin hydrochloride monotherapy group and telmisartan monotherapy group ($P < .05$).

Mitochondrial membrane potential among the three groups

Figure 5 displays no difference in MMP ratio among the three groups prior to therapy ($P > .05$). After therapy, MMP ratio was elevated in all groups, and it was higher in the combination therapy group than metformin hydrochloride monotherapy group and telmisartan monotherapy group ($P < .05$).

Correlation between PINK1 and clinical indicators in patients with diabetic nephropathy

Figure 6 displays that PINK1 was positively correlated with eGFR, while negatively correlated with UACR and SCr in all groups ($P < .001$).

Correlation between PINK1 and inflammatory factors in patients with diabetic nephropathy

Figure 7 reveals that PINK1 has a positive correlation with IL-2 and a negative correlation with TNF-α and IL-6 in all groups ($P < .001$).

DISCUSSION

Diabetic nephropathy is one of the common complications of diabetes mellitus, and its pathogenesis is still unknown.[16] Clinical intervention can only effectively mitigate the

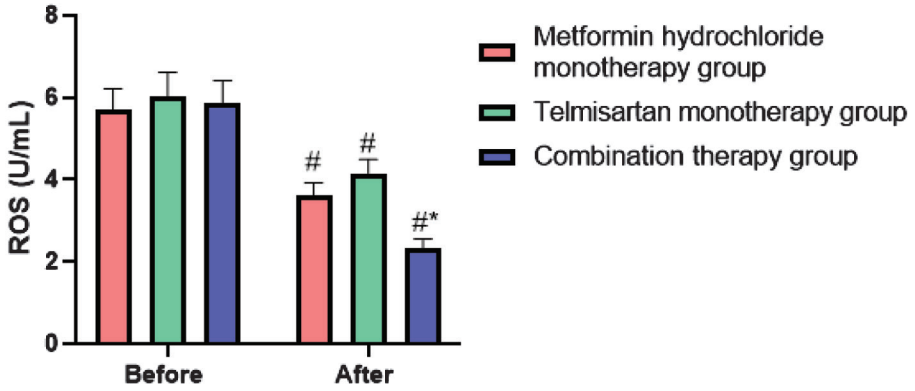

Figure 4. ROS level among the three groups. $^{\#}P < .05$, compared with before therapy. $^{*}P < .05$, compared with metformin hydrochloride monotherapy group and telmisartan monotherapy group.

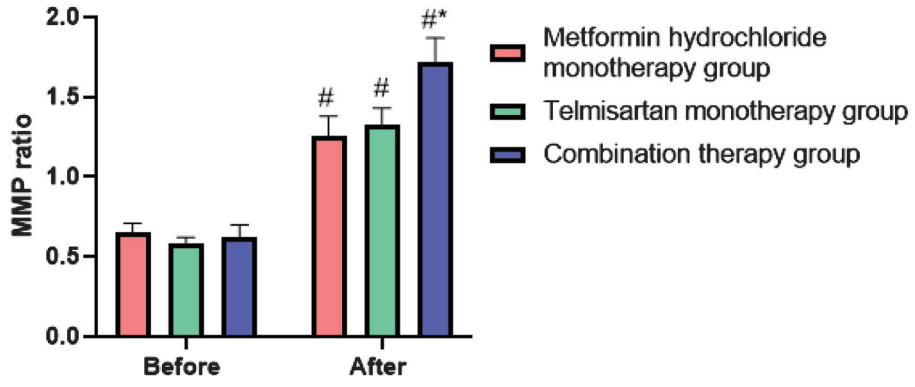

Figure 5. Mitochondrial membrane potential among the three groups. $^{*}P < .05$, compared with metformin hydrochloride monotherapy group and telmisartan monotherapy group.

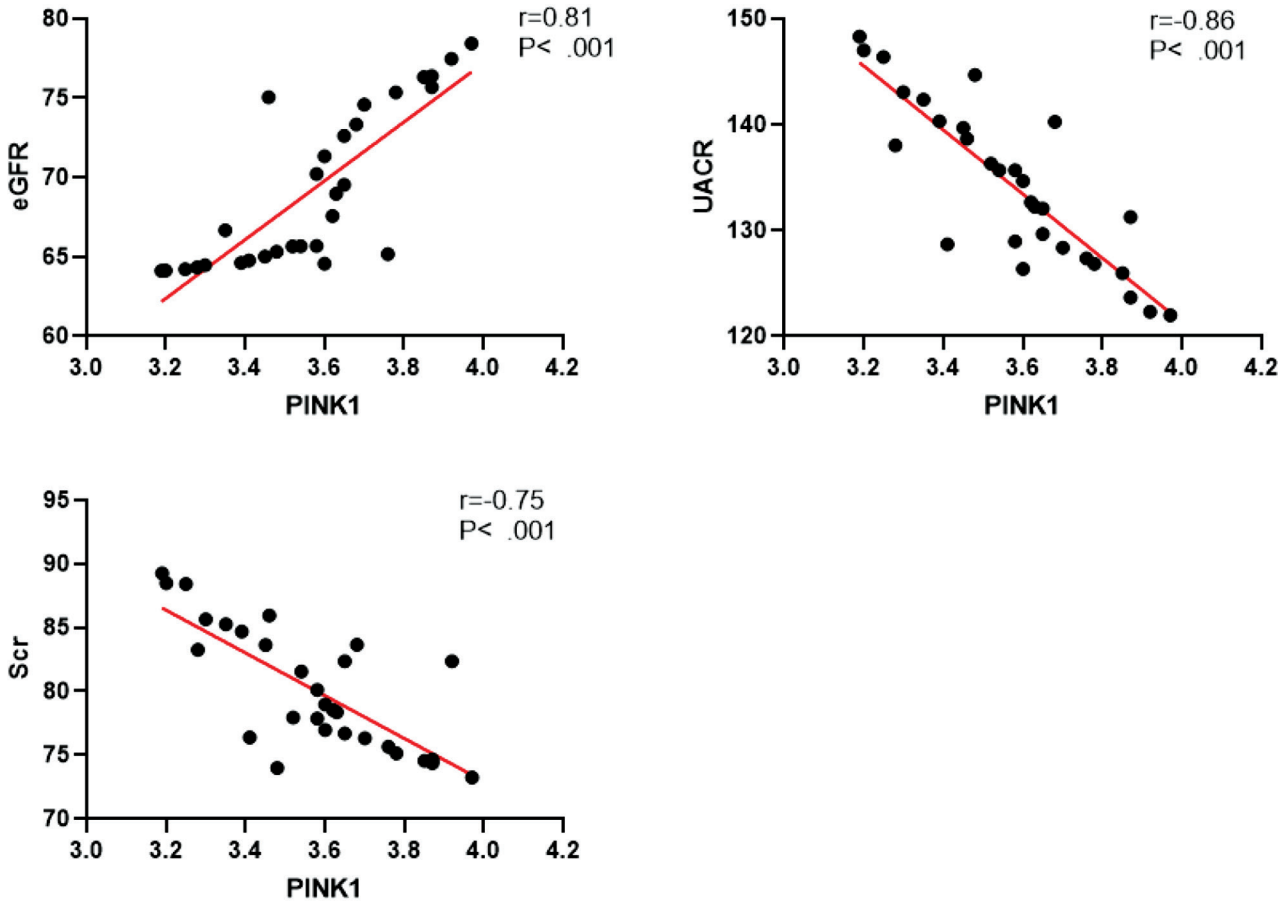

Figure 6. Correlation between PINK1 and clinical indicators in patients with diabetic nephropathy.

advancement of the disease by managing patients' blood glucose, blood pressure, and lipid levels. However, it cannot fundamentally stop the course of the disease.[17]

Telmisartan is a fast-acting specific angiotensin II receptor antagonist that, in addition to its use in the treatment of essential hypertension, can also be applied in the clinical treatment of diabetic nephropathy. The drug primarily stimulates the production of mesangial and vascular wall muscle cells, leading to the formation of negatively charged sulfate heparin-like substances. This inhibits the filtration of urine protein, accelerates the recovery of the charge barrier, decreases the diameter of the filter holes in the glomeruli, and enhances the permeability of the glomerular filtration membrane.[18,19]

Metformin is prescribed either as a standalone treatment or in combination with other medications for the management of diabetes mellitus.[20] Metformin hydrochloride tablets can be administered in patients with type II diabetes mellitus whose blood sugar cannot be controlled through dietary restriction especially those who are obese and have insulin resistance.[21] Metformin hydrochloride tablets have the potential to reduce weight and control hyperinsulinemia in addition to their hypoglycemic effect.[22] In some patients with poor efficacy of sulfonylureas, the effect of metformin hydrochloride tablets in combination with sulfonylureas, α-glucosidase inhibitors or thiazolidinediones hypoglycemic drugs is better, compared with that of single use.[23]

Glomerular endothelial cells are rich in mitochondria due to their consistently high level of energy expenditure.[24] In the initial stages of kidney disease, metabolic alterations that disrupt mitochondrial homeostasis, including mitochondrial dysfunction and impaired energy metabolism, are typical. These alterations are accompanied by a cascade of mitochondrial changes, including fragmented morphology, increased ROS

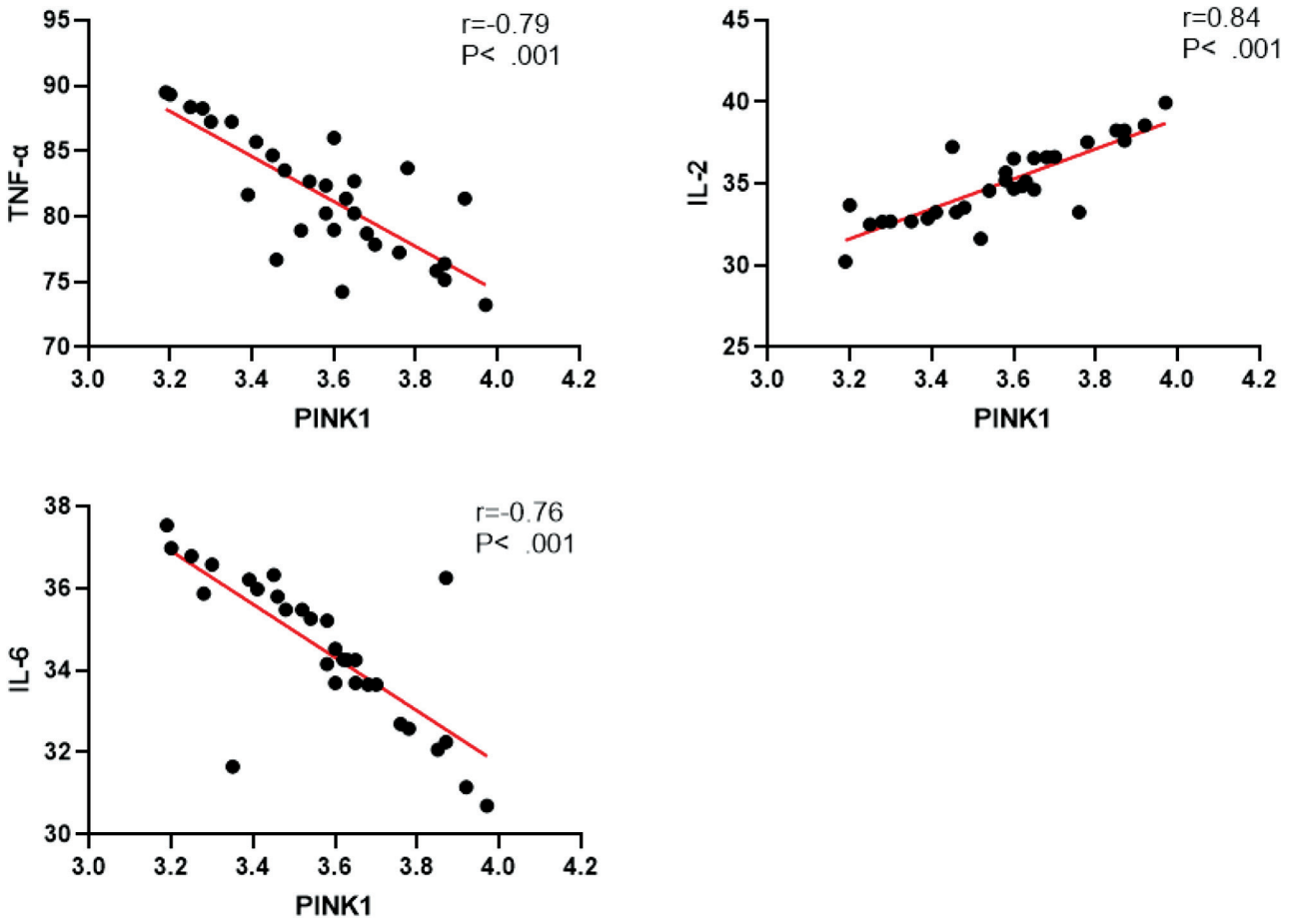

Figure 7. Correlation between PINK1 and inflammatory factors in patients with diabetic nephropathy.

production, and loss of mitochondrial membrane potential. Ultimately, this results in podocyte loss and detachment, loss of the podocyte process, and glomerular filtration barrier disruption and proteinuria.[25]

PINK1 functions as a critical regulator of mitochondrial function, and many reports have suggested that many drugs attenuate the progression of diabetic nephropathy through the regulation of PINK1-mediated mitochondrial function.[26] For example, Huangkui capsule relieved diabetic kidney disease through inducing mitophagy mediated by STING1/PINK1 signaling.[27] Dioscin also relieved diabetic nephropathy via improving mitophagy and mitochondrial fission/fusion mediated by PINK1.[28] Recent studies have discovered that various cytokines-induced inflammation also plays a crucial role in the development of diabetic nephropathy.[29] Various inflammatory factors amplify the inflammatory effect through autocrine and paracrine pathways, causing inflammatory cascade reactions, and induce oxidative stress in the body, directly damage glomerular endothelial cells, increase monocyte adhesion and infiltration to vascular endothelium, and release various cytokines to exacerbate the inflammatory process and kidney injury.[30]

In our study, the results showed that the PINK1 expression level was higher, and the ROS level was lower in the combination therapy group after therapy than in the metformin hydrochloride monotherapy group and telmisartan monotherapy group. The improvements in renal function indices and inflammatory factors were more apparent in the combination therapy group than in the metformin hydrochloride monotherapy group and telmisartan monotherapy group. Additionally, the MMP ratio was higher in the combination therapy group than in the metformin hydrochloride monotherapy group and telmisartan monotherapy group. All these findings suggest that the combination treatment of metformin hydrochloride and telmisartan

effectively promote PINK1 expression level, reduce inflammatory response and ROS level, as well as maintained MMP, which is consistent with previous studies.[31-33]

Besides, our study revealed that PINK1 was negatively correlated with UACR, SCr, TNF-α and IL-6, while positively correlated with eGFR and IL-2, suggesting that PINK1 expression may be involved in inhibiting the occurrence and development of diabetic nephropathy. Similarly, Sun *et al.* have shown that PINK1 exerts protective effects in diabetic nephropathy by attenuating mitochondrial dysfunction and necroptosis.[34] Also, Irena Audzeyenka *et al.* have pointed that PINK1 has an essential role in insulin signaling and maintaining proper permeability in podocytes, which may be a potential therapeutic target in the treatment of diabetic nephropathy.[35]

Nevertheless, this work still has several limitations. Our results may not be very convincing because of the small number of patients we surveyed for this study. In addition, although our work has done some research on the protective effects of metformin hydrochloride and telmisartan combination therapy and PINK1 in diabetic nephropathy, the protective mechanism has not been studied deeply enough. Therefore, in the future studies , we would focus on the protective mechanism of diabetic nephropathy of PINK1 by combination therapy and through animal and cell models.

CONCLUSION

In conclusion, PINK1 is expressed at low levels in patients with diabetic nephropathy. PINK1 expression is strongly associated with the inhibition of disease progression and may serve as a potential biological marker for clinical diagnosis. PINK1 exerts a protective role in diabetic nephropathy patients under the combination therapy of metformin hydrochloride and telmisartan, which may provide a novel insight of treatment of diabetic nephropathy.

ETHICAL APPROVAL

The study protocol was approved by the Ethics Committee of Longquan City People's Hospital.

CONFLICTS OF INTEREST

The authors declare no competing interests.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

Zhihong Zeng contributed to the study design. Experiments, data collection and analysis were performed by Dongmei Xu, Feng Cai and Jiexi Hu. The first draft of the manuscript was written by Dongmei Xu, and all authors commented on previous versions of the manuscript. All authors read and approved the final submitted manuscript.

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