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Original Research Article

Effect of combined treatment with benazepril and spironolactone on diabetic nephropathy and serum levels of IL-6, CRP and TNF-α

Xiaoxing Duan, Liying Zhang, Xue Pan, Dongying Shi*
Department of Nephrology, The Affiliated Hospital of Inner Mongolia Medical University, Inner Mongolia 010050, China

For correspondence:* ***Email:* *s90m6m@163.com*

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Abstract

Purpose: *To study the therapeutic effect of a combination of benazepril and spironolactone on diabetic nephropathy patients, and also to determine the influence of the combined treatment on serum IL-6, CRP and TNF-α.*
Methods: *100 diabetic nephropathy patients admitted to The Affiliated Hospital of Inner Mongolia Medical University from April 2019 to October 2020 were randomly chosen and divided into groups E and F by drawing lots (n = 50 each). Group E received benazepril, while group F received a combined treatment of benazepril and spironolactone. Therapeutic efficacy, incidence of adverse drug reactions, and renal function after treatment, as well as IL-6, CRP and TNF-α levels in serum pre- and post-treatment, were determined. Fasting blood glucose (FBG) levels were also measured pre-treatment, and at 7 days and 14 days post-exposure to drugs.*
Results: *Therapeutic efficacy and renal functions were significantly better in group F than in group E, while post-treatment incidence of adverse drug reactions, and expression levels of CRP, IL-6, and TNF-α levels were significantly reduced in group F, relative to group E ($p < 0.05$). Following treatment, the inflammatory factor levels were decreased in both groups.*
Conclusion: *The combination of benazepril and spironolactone produces higher treatment effect in diabetic nephropathy patients than those that received benazepril only. The combined treatment is recommended in the management of diabetic nephropathy patients.*

Keywords: *Benazepril, Spironolactone, Diabetic nephropathy, Inflammatory factors, Fasting blood glucose*

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INTRODUCTION

Diabetic nephropathy is a serious complication of diabetes, and long-term high blood glucose level in patients causes severe pressure on the kidneys, leading to renal dysfunction [1-3]. The main clinical manifestations of diabetic nephropathy are proteinuria, edema, hypertension and anemia. Renal failure, which may occur in severe cases, poses a great threat to the lives of the patients. Therefore, patients with early diabetic nephropathy should seek medical treatment and receive corresponding treatment measures [4-6].

Benazepril, a drug which is hydrolyzed into angiotensin converting enzyme, is often used to treat dilated arteries and veins. Besides, it stimulates increase in plasma renin activity, and it is widely applied in clinical treatment of diseases such as hypertension and heart failure. Spironolactone, a diuretic which is regularly used in clinics, is often used in treating patients with hypertension, hypokalemia and edema [7-9].

It has been reported that the combined use of benazepril and spironolactone markedly improved the renal function of patients. Thus, a combination the two drugs may be used in the treatment of renal dysfunction.

The present study was carried out to investigate the effectiveness of benazepril-spironolactone combined treatment on diabetic nephropathy patients, and its influence on blood levels of some inflammatory factors. In essence, diabetic nephropathy patients (research subjects) were treated with combination of benazepril and spironolactone, or with benazepril only. Therapeutic efficacy, incidence of adverse reactions, post-treatment renal function, and levels of inflammatory factors (pre- and post-treatment), as well as FBG levels before treatment, and 1 week and 2 weeks after treatment, were determined and compared between the two categories of patients.

METHODS

Patient characteristics

One hundred diabetic nephropathy patients admitted to The Affiliated Hospital of Inner Mongolia Medical *University* from April 2019 to October 2020 were randomly chosen, and divided equally into group E and group F by drawing lots. Subjects in group F ranged in age from 36 to 66 years, while patients in group E were aged 35 to 67 years. The two groups of subjects were comparable.

Ethical approval

The study was approved by the ethics committee of The Affiliated Hospital of Inner Mongolia Medical *University (approval no. 20190239), and it was done in line with* the declaration of Helsinki as revised in 2013 [10]. All patients voluntarily took part and submitted written consent.

Inclusion/exclusion criteria

Inclusion criteria

The following categories of patients were enrolled in this research: patients with clinical manifestations of diabetic nephropathy, patients aged a minimum of 18 years, patients with no history of drug reactions, drug misuse and unhealthy habits, and those free from other organic diseases.

Exclusion criteria

Patients with impaired consciousness, those who were uncooperative during the study, subjects with kidney disease, and non-diabetic patients, were not included.

Treatments

Group E received benazepril. The patients in group E were given 10 mg of benazepril tablets (Chengdu Di Ao Pharmaceutical Group Co. Ltd, NMPA approval no. H20053390; specification: 10 mg) once a day for one month. Thereafter, the curative effect was determined.

Group F received combination of benazepril and spironolactone. The dose of benazepril given was the same as that in group E. In addition, the patients were given 20 mg of spironolactone (Jiangsu Chia Tai Fenghai Pharmaceutical Co. Ltd, NMPA approval no. H32020077; specification: 20 mg) once a day for a duration of one month, and the curative effect of the drug combination was determined.

Table 1: Characteristics of patients (mean ± SD)

Variable	Group F	Group E	X^2/t	*P*-value
Gender (male/female)	23/27	26/24	0.36	0.55
Age (years)	52.36±6.67	52.17±6.39	0.15	0.88
Height (cm)	168.05±10.01	168.79±10.37	0.36	0.72
Weight (kg)	70.45±5.83	70.39±5.64	0.05	0.96
Course of disease (months)	5.68±1.69	5.72±1.73	0.12	0.91
Smoking history (years)	4.26±1.33	4.27±1.38	0.04	0.97
Drinking history (years)	7.96±1.38	7.52±1.22	1.69	0.09
Hypertension (cases)	24	25	0.04	0.84
Hyperlipidemia (cases)	18	20	0.17	0.68

Determination of indices

Therapeutic effectiveness (TE)

Clinical treatment effects were measured and compared between the two groups. Treatment was deemed *markedly effective* under the following conditions: urinary protein excretion was decreased to normal level or decreased by more than 50 %, proteinuria in 24 hours decreased by more than 50 %, blood glucose and glycosylated hemoglobin returned to normal or decreased by more than 33 %, renal function became normal, and the signs and symptoms were significantly reduced. Treatment outcome was *effective* under the following conditions: renal function returned to normal, proteinuria in 24 hours decreased by less than 50 %; urinary protein excretion, blood glucose and glycosylated hemoglobin were decreased by less than corresponding values for *markedly effective*, and signs and symptoms were reduced. However, if renal function, proteinuria in 24 hours, and signs and symptoms were not improved, or if they became worsened, the treatment was regarded as *ineffective*.

$$TE\ (\%) = \frac{(ME + E)}{T} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where: *TE* = therapeutic effectiveness; *ME* = number of markedly effective cases; *E* = number of effective cases; *T* = total number of patients.

Post-treatment incidence of unwanted reactions were statistically analyzed for groups E and F.

Renal function indices

Early morning fasting venous blood (5 ml) and urine were collected from both groups for determination of urea nitrogen, urinary albumin, serum creatinine and creatinine clearance. Normal creatinine clearance values range from 80 - 120 mL/min. Lower levels are usually indicative of impaired renal function. The normal serum creatinine is less than 133 µmol/L, and renal function decreases with increases in serum creatinine. Moreover, the normal urea nitrogen is less than 7.9 mmol/L, with higher levels indicating decreases in renal function. For albumin, the normal urinary levels are in the range of 20 - 80 mg/24 h, with higher urinary albumin indicating poorer renal function [10-12].

Serum inflammatory factor indicators

ELISA was used to measure the levels of various serum inflammatory factor indicators after treatment in both groups. These were interleukin-6 (IL-6), C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α). The assay kits were purchased from Nanjing Senbeijia Biotechnology Co. Ltd. The assays were done strictly in accordance with the kit instructions.

Blood glucose levels

Fasting blood glucose (FBG) levels in both groups were determined before treatment, 1 week after treatment, and 2 weeks after treatment. The results were compared with the normal FBG value (not less than 5.6 mmol/L).

Statistical analysis

The selected data processing software used was SPSS 20.0, while GraphPad Prism 7 was used for graphics. Measurement results are presented as mean ± SD, and were compared with *t*-test. Counted data are expressed as numbers and percentages [n (%)], and were compared with χ^2 test. Values of $p < 0.05$ implied significant differences.

RESULTS

Therapeutic efficacy

Treatment effectiveness was markedly better in group F than in group E.

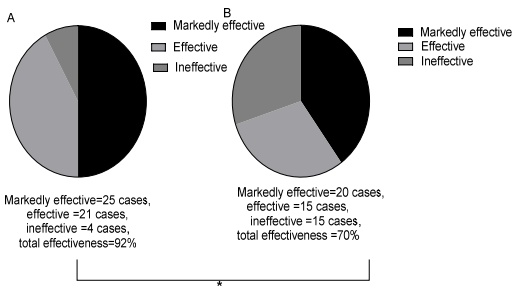

Figure 1: Comparison of therapeutic efficacy between E & F. 1A shows the therapeutic efficacy of group F, in which the population of patients in markedly effective, effective, and ineffective categories were 25, 21 and 4, respectively, accounting for total effectiveness of 92 %. 1B shows the therapeutic efficacy of group E, in which the number of patients in markedly effective, effective and ineffective categories were 20, 15 and 15, respectively, resulting in total effectiveness of 70 %. *$P < 0.05$, therapeutic efficacy of group E vs therapeutic efficacy of group F.

Incidence of adverse reactions

Comparison of the incidence of adverse drug reactions showed that the incidence was lower in group F, relative to group E.

Table 2: Incidence of adverse reactions

Group	Increased serum potassium	Cough	Rebound of urinary protein level	Total incidence (%)
F	2	3	1	12
E	4	4	8	32
χ^2				5.83
P-value				0.02

Table 3: Values of renal function (mean ± SD)

Group	Creatinine clearance (mL/min)	Serum creatinine (μmol/L)	Urea nitrogen (mmol/L)	Urinary albumin (mg/24h)
F	96.38±4.49	84.55±5.67	5.58±0.31	65.65±5.22
E	77.90±4.21	121.97±9.90	7.01±0.69	76.06±6.70
t	21.23	23.19	13.37	8.67
P	<0.001	<0.001	<0.001	<0.001

Renal function after treatment

After treatment, the results of renal function significantly better in group F than in group E *(p* < 0.05).

Serum levels of IL-6, CRP and TNF-α

The post-treatment levels of these inflammatory factors were markedly lower in group F than in group E *(p* < 0.05). However, before treatment, no significant difference was found in their levels between the two groups *(p* > 0.05).

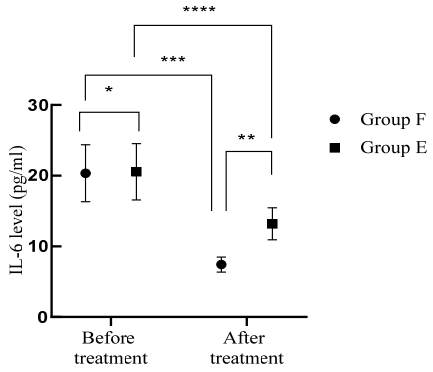

Figure 2: Comparison of serum IL-6 levels between E & F

In group F, the IL-6 level was 20.34 ± 4.02 pg/mL before treatment, and 7.43 ± 1.06 pg/mL after treatment. In group E, the IL-6 level was 20.55 ± 3.98 pg/mL before treatment, and 13.20 ± 2.27 pg/mL after treatment. **P > 0.05,* IL-6 level in E before treatment vs IL-6 level in F before drug exposure. *** P < 0.05,* IL-6 level in E post-treatment vs IL-6 level in F after treatment; **** p < 0.05,* IL-6 level in F before treatment vs IL-6 level in F after treatment; ***** p < 0.05,* IL-6 level in E before treatment vs IL-6 level in E after treatment.

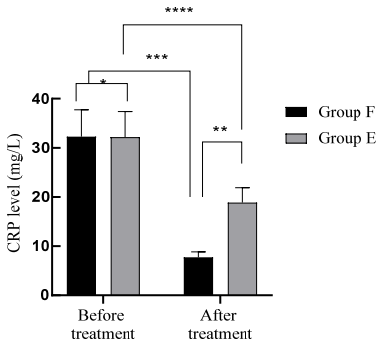

Figure 3: Comparison of serum CRP levels between E & F. In group F, the CRP level was 32.28 ± 5.47 mg/L before treatment, and 7.75 ± 1.09 mg/L after treatment. In group E, the CRP level was 32.19 ± 5.20 mg/L before treatment, and 18.88 ± 3.00 mg/L after drug exposure. **P > 0.05,* CRP levels in groups E and F before treatment. ***P < 0.05,* CRP levels in groups E & F after treatment; **** p < 0.05,* CRP levels in group F before and after treatment; ***** p < 0.05,* CRP levels in group E before and after treatment

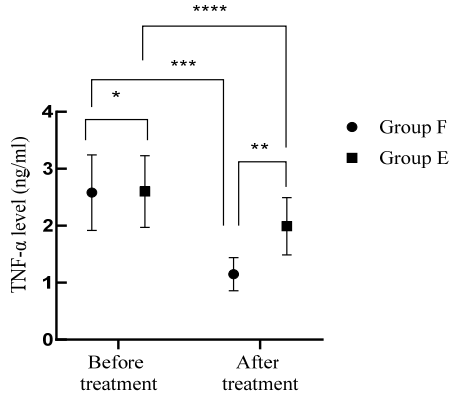

Figure 4: Comparison of serum TNF-α levels between E & F. In group F, the TNF-α level was 2.58 ± 0.66 ng/mL before treatment, and 1.15 ± 0.29 ng/mL after treatment. In group E, the TNF-α level was 2.60 ± 0.63 ng/mL before treatment, and 1.99 ± 0.50 ng/mL after treatment. **P > 0.05,* TNF-α level in group E before

treatment vs TNF-α level in group F before drug exposure; ** $p < 0.05$, TNF-α levels in groups E and F after treatment; *** $p < 0.05$, TNF-α level in group F before treatment vs the TNF-α level in group F after treatment; **** $p < 0.05$, TNF-α level in group E before treatment vs TNF-α level in group E after treatment

Fasting blood glucose levels

Comparison of the fasting blood glucose levels showed no obvious differences between the two groups ($p > 0.05$).

Table 4: Comparison of fasting blood glucose levels (mean ± SD, mmol/L, n = 50))

Group	Before treatment	1 week after treatment	2 weeks after treatment
F	6.32±0.54	5.98±0.37	6.01±0.41
E	6.24±0.55	6.05±0.40	6.10±0.40
t	0.734	0.908	1.111
P-value	0.465	0.366	0.269

DISCUSSION

In the treatment of patients with diabetic nephropathy, the first step is to control and maintain the blood glucose level at a safe range, and then stabilize glycosylated hemoglobin indexes, so as to effectively control the risk of other complications. The main clinical manifestations of diabetic nephropathy are proteinuria and edema of the limbs. If the disease is not well controlled and treated for a long time, the patients will be at risk of renal failure and uremia. Once uremia due to diabetic nephropathy sets in, the chances of cure are markedly decreased, resulting in the need for long-term hemodialysis in order to maintain life [13-15].

Generally speaking, the kidney is an organ that is crucial for filtration and metabolism. It filters off and discharges excess water from the body, thereby ensuring homeostasis in electrolytes such as potassium and sodium [16-18]. If the renal function of patients with nephropathy decreases, and normal metabolism is impaired, it may easily lead to complications e.g., infection, which will make the treatment of nephropathy more difficult. Benazepril and spironolactone are drugs clinically used for treating hypertension and diuresis, often in patients with renal dysfunction [19-21].

It has been reported that combined use of benazepril and spironolactone improves the effect of treatment on nephropathy. In this study, diabetic nephropathy patients were treated with combination of benazepril and spironolactone, or with benazepril alone. Therapeutic efficacy, incidence of adverse reactions, renal function after treatment, serum levels of CRP, TNF-α and Il-6, as well as fasting blood glucose were measured and analyzed. This study showed that the therapeutic efficacy and renal function after treatment were markedly better in the patients given combined treatment. The indicators of renal function are creatinine clearance, serum creatinine, urea nitrogen and urinary albumin. Abnormal renal function in patients is directly reflected in the abnormality of various indexes. Thus, changes in renal function can be judged by the results of the various indexes measured [22-24]. The results indicated that the combined treatment with benazepril and spironolactone significantly improved the renal function of diabetic nephropathy patients, and produced a better treatment effect than single drug therapy.

The incidence of adverse reactions, and serum levels of inflammatory factors after treatment in group F were markedly lower than those in group E. Blood levels of inflammatory factors in both groups were markedly decreased, relative to corresponding pre-therapy levels. Diabetic nephropathy patients are prone to infection, leading to increased inflammatory factor levels. This study has shown that the expression levels of serum inflammatory factors in diabetic nephropathy patients treated with combination of benazepril and spironolactone decreased significantly, and the decrease was more obvious than that in patients treated with benazepril. This indicates that the combined therapy regulated the inflammatory status in patients. In addition, measurement of the fasting blood glucose levels before and after treatment revealed no significant increases or decreases under the influence of the drugs.

Consistent with data obtained here, it has been reported that the combined use of benazepril and spironolactone produced a good effect on diabetic nephropathy patients as evidenced in improvement of renal function, and marked reduction of the risk of other related complications. Thus, data obtained in this investigation reflect the actual effect of the combined treatment.

CONCLUSION

The findings of this study show that the combined use of benazepril and spironolactone was an effective therapy for diabetic nephropathy patients. In addition, the combined treatment improves renal function and lowers the levels of inflammatory factors in patients. Thus, this

treatment strategy may be beneficial for the management of diabetic neuropathy patients.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. XD conceived and designed the study, and drafted the manuscript. LZ and XP collected, analyzed and interpreted the experimental data. DS revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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