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Research Article
Therapeutic Efficacy of Combined Therapy with Breviscapine and Methylcobalamin in Diabetic Peripheral Nephropathy Management

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Abstract

Background and Objective: Breviscapine considered to be an active ingredient of flavonoids and able to dilate the blood vessels, reduce vascular resistance, increase blood flow, improve microcirculation and suppress the aggregation of platelets. This study was assessed the therapeutic effect of breviscapine in combination with methylcobalamin in the management of diabetic peripheral nephropathy. **Materials and Methods:** The sample size of 300 subjects was taken and subdivided equally into 2 groups. Treatment group subjects were given a breviscapine oral dosage of 20 mg/day and a tablet of Vitamin B which further contained the 2 mg dL^{-1} of folic acid and 1 mg dL^{-1} of methylcobalamin. The therapy was given for continuous 12 months and followed up for 36 months. Another group had taken as a placebo group and received a dosage of 1 mg dL^{-1} of methylcobalamin only. Glomerular filtration rate (GFR) was taken as primary level outcome. **Results:** Increased radionuclide GFR was 6.2 mL min^{-1}/1.73 m^2 in the treatment group at 18 months and 10.8 mL min^{-1}/1.73 m^2 at 36 months and decreased to -3.1 mL min^{-1}/1.73 m^2 at 18 months and -5.7 mL min^{-1}/1.73 m^2 at 36 months in the placebo group. So, a higher level of radionuclide GFR at 18 months and at 36 months was found in the treatment group when compared with the baseline level and placebo with significant p-value of less than 0.05. **Conclusion:** So it was concluded that the breviscapine improves the diabetic nephropathy sign and symptoms and also improves the quality of life of the subjects.

Key words: Breviscapine, methylcobalamin, glomerular filtration, plasma homocysteine, diabetic nephropathy

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetic nephropathy is a progressive disease and more prevalent in the developing countries[1] and breviscapine is a purified flavonoid extract from the hand-mazz plant species available in Yunnan China. The primary component of the breviscapine is Scutellarin, which accounts for 92% of total extract and other flavonoids account[2,3] for only 5%. The Chinese authors published many articles about the wide coverage of the pharmacological benefits of this extract that included the improvement in the microcirculation, dilating the blood vessels and reducing the viscosity of blood. So, they concluded that this medicine could be used in the management of cardiovascular and cerebrovascular disease such as myocardial infarction, cerebellar infarction and stroke[4,5].

In addition in cardiovascular effects, breviscapine is considered to be a strong antioxidant agent and possess the Protein Kinase C inhibitor property, which further improves the renal function and decreases the albuminuria in chronic end renal disease[6,7]. So, the role of this medicine in diabetic nephropathy should be analyzed. Some of the authors had done the pilot study to reveal the effects of breviscapine on diabetic nephropathy[8], however, not a single author had revealed the proper treatment protocol and follow up period.

One of the published report revealed that the breviscapine improves the renal fibrosis at the interstitial level by UUO through the alpha- SMA and TGF0 Beta 1 expression and providing the renoprotective effects. The study also revealed that the breviscapine affects the sodium protein expressions and further improves the renal function[9].

Breviscapine can also dilate capillaries, reduce vascular resistance and platelet aggregation, produce anti-coagulation effects, scavenge free radicals and improve microcirculation[10]. Recent studies concluded that there was strong association between the high level of plasma homocysteine level and risk of diabetic nephropathy, retinopathy and other vascular diseases. Literature survey concluded that the Vitamin B therapy helps in lowering the total plasma homocysteine levels and further improves the function of endothelial cells[11]. The main objective of the study was to examine the protective effect of breviscapine in combination with methylcobalamin in the management of diabetic peripheral nephropathy.

MATERIALS AND METHODS

This study had been done in the single hospital, parallel group, open and full randomized trial in the Nanchong central hospital, China. The diagnosis of type 2 diabetes mellitus had been made as per the criteria designated by the American dental association[12]. The study had been started from Jan, 2014 after taking the ethical approval from the Institution Research cum Thesis board with allotted No. Nan/Uni/CT6/2014. The first sample had been taken on dated 12th January, 2014 and completed the sample size of 30 November, 2017. The total duration of the study was 3 years 10 months. All the study sampling, diagnosis and treatment had been done in Nanchong central hospital, Nanchong.

Inclusion and exclusion criteria: Patients had T2DM with >11% glycosylated hemoglobin and 7% mmol L^{-1} fasting blood glucose level or oral GTT 2h postprandial level more than 11 mmol L^{-1} had been taken as inclusion criteria. Those patients with T2DM were excluded when renal diseases attributable to other causes[8].

Distribution of sample and allotment to groups: The total sample size was comprised of 300 subjects and divided into 1:1 ratio with matched age and gender. Treatment group subjects were given a breviscapine with oral dosage of 20 mg/day and a tablet of Vitamin B which further contained the 2 mg dL^{-1} of folic acid and 1 mg dL^{-1} of methylcobalamin. The therapy was given for continuous 12 months and followed up for 36 months. The other group taken as a placebo group, this group received dosage of 1 mg dL^{-1} of methylcobalamin only. Each and every subject was assessed at baseline, after 18 months and subsequently after 36 months. To assess whether the subjects taking the medication properly, plasma total homocysteine levels, vitamin B12 levels and total serum folate levels were also assessed.

Assessing the primary level outcome

(A) Assessing the GFR levels by various approaches: The effect and outcome of therapy was assessed by the estimating glomerular filtration rate and taken as the primary level outcome[13]. The 99 Tech-DTPA techniques were taken as first approach for assessing the radionuclide GFR at baseline, 18 and 36 months. Creatinine levels were taken as 2nd approach to measure the GFR. The MDRD formula i.e., 4 variable modification of diet had also been used for assessing the values of GFR and taken as the 3rd approach. The average of 3 values had been taken for each method at the 30 min interval per subject visit[13]. The values were evaluated by the famous nephrologists through the blinded approach.

Radionuclide assay was taken as the first priority to calculate the GFR levels, if radionuclide GFR values were unavailable then the second priority was given to creatinine clearance. If both the methods were unavailable to calculate the GFR then MDRD formula was used to calculate the GFR levels. When all the methods were not available to calculate the GFR, the multiple imputations with the analysis of predictive model based method was used to calculate the GFR. Out of total 300 subjects, 10 subjects did not return after the post randomization period to measure the GFR efficacy. So, the analysis had been done in total of 290 subject's i.e., 145 in the treatment group and 145 in the placebo group[13].

(B) Urine albumin ratio: Urine albumin ratio also had been taken to measure the primary level outcome. According to the ratio of urine albumin/urine, subjects had normal ratio: Normoalbuminuria group having the ratio of UACR up to 30 mg b^{-1}. Subjects had raised ratio: Microalbuminuria group having the ratio of UACR more than 30-300 mg b^{-1}. Subjects had a very high ratio: Macroalbuminuria group having the ratio of UACR more than 300 mg b^{-1}. The levels of UACR were measured by the ELISA assay[8].

Assessing the secondary and tertiary level outcomes: The committee of blinded reviewers had been constituted at institute level to assess the secondary and tertiary level outcomes such as myocardial infarction, cardiac angioplasty, amputation from the vascular diseases of peripheral origin and cause of mortality. Any harmful changes that were analyzed during the trial period were considered under the category of adverse effects[14,15].

Analysis of other parameters: The oxidase method was used for the examination of fasting blood glucose level (FBG) and triglyceride level (TG). The LDL, alanine transaminase (ALT), BUN and blood uric acid (UA) were measured by the ELISA enzyme colorimetry[15].

Statistical analysis: Statistical analysis had been done by using the SAS software version 21.0 and imputation on multiple basis was analyzed by SOLAS software. The results were assessed in the treatment groups by least square means, standard deviations (SD), standard error (SE) and 95% of the confidence interval (95%CI). The analysis at primary level outcome between the treatment group and the placebo group was assessed by one way ANOVA. Univariate cox hazard model of regression, hazard ratio and confidence interval at 95% was calculated to assess the secondary and tertiary level outcome. Kaplan meier analysis had also been taken to calculate the risk outcome for the treatment groups. The p-value of less than 0.05 was taken as statistically significant level.

RESULTS

The average age of the total sample size was 61.2+/-2.5 years. The subjects had given the follow up in median interquartile range of 1.1-32.2 months. Out of total sample size, 18 subjects (6.02%) did not give follow up (Table 1).

Analysis of urine albumin ratio: A statistical significant decline in urine albumin ratio was observed in the treatment group at baseline and at 36 months with significant p-value of less than 0.05. The placebo group patients did not show the decline of UACR levels at baseline and at 36 months (Table 1).

Analysis of urinary parameters: At follow up of 18 and 36 months, the subjects in the treatment group revealed lesser levels of fasting blood glucose and decrease in diastolic blood pressure as compared to the placebo groups. The triglyceride levels and BUN was decreased was in the treatment group subjects as compared to placebo group with statistical significant p-value of less than 0.05 (Table 1).

Analysis of GFR levels

Radionuclide GFR: The radionuclide GFR revealed that the increased GFR was 6.2 mL min^{-1}/1.73 m^2 in the treatment group at 18 months and 10.8 mL min^{-1}/1.73 m^2 at 36 months. GFR level was decreased to -3.1 mL min^{-1}/1.73 m^2 at 18 months and -5.7 mL min^{-1}/1.73 m^2 at 36 months in the placebo group (Table 2). The MDRD GFR: Increased GFR was 4.5 mL min^{-1}/1.73 m^2 in the treatment group at 18 months and 10.5 mL min^{-1}/1.73 m^2 at 36 months and decreased to -4.2 mL min^{-1}/1.73 m^2 at 18 months and -5.9 mL min^{-1}/1.73 m^2 at 36 months in the placebo group (Table 2). The results defined that the treatment group patients had a much greater increase level of radionuclide GFR and MDRD GFR rate as compared with the placebo group with significant value of less than 0.05 at 18 and 36 months (Table 2).

Table 1: Baseline characteristics and 36 months characteristics of treatment group and placebo group

Characteristics	Treatment group at baseline	Placebo group at baseline	Treatment group at 36 months	Placebo group at 36 months	Total values/ Percentage	p-value
Number	150	150	145	145	290	-
Follow up (subjects in number)	-	-	137	135	272	-
Subjects who did not returned for follow up			8 (5.83%)	10 (7.40%)	18 (6.02%)	
Median Interquartile range of follow up (IQR) in months	-	-	1.1-32.2	1.4-33	1.1-32.2	-
Age (years)	60.2+/-3 years	63.7+/-2 years	60.2+/-3 years	63.7+/-2 years	61.2+/-2.5 years	-
Body weight (kg)	69.2+/-7.7	72.4+/-6.3	74.1+/-4.5	69.3+/-5.2	-	0.20
Insulin levels	180.2+/-4.5	203.1+/-5.4	135.1+/-4.7	180.12+/-4.7	-	<0.05
HbA1c levels (%)	8.54+/-1.1	9.54+/-1.2	6.89+/-1.1	8.12+/-0.9	-	<0.05
Triglyceride level (mg dL^{-1})	178.67+/-21.1	188.78+/-27.4	98.2+/-13.2	178.3+/-1.9	-	For placebo p>0.05 For treatment p<0.05
UACR (ng mg^{-1})	13.2+/-2.1	14.3+/-1.7	5.3/-1.2	14.1+/-1.1	-	For placebo p>0.05 For treatment p<0.05
Fasting blood glucose (FBG) (mmol L^{-1})	13.12+/-1.2	12.34+/-2.2	7.32+/-2.1	11.98+/-1.9	-	For placebo p >0.05 For treatment p<0.05
Diastolic blood pressure (DBP) (mm hg^{-1})	100+/-4	105+/-3	82+/-2	100+/-5	-	For placebo p >0.05 For treatment p<0.05
BUN (md dL^{-1})	83.41+/-3.2	85.7+/-1.9	32.2+/-2.6	75.2+/-2.1	-	For placebo p >0.05 For treatment p<0.05

UACR: Urine albumin to creatinine ratio, BUN: Blood urea nitrogen

Table 2: Mixed linear models at baseline and at endpoint for treatment group and placebo group to assess the GFR levels and total plasma homocysteine levels

Parameters	Baseline Score	18 months	36 months	p-value
Radionuclide GFR (mL min^{-1}/1.73 m^2)				
Treatment group	54.7	+6.2	+10.8	<0.05
Placebo group	53.7	-3.1	-5.7	0.32
MDRD GFR (mL min^{-1}/1.73 m^2)				
Treatment group	58.1	+4.5	+10.5	<0.05
Placebo group	58.1	-4.2	-5.9	0.98
Plasma total homocysteine level (µmol L^{-1})				
Treatment group	15.6	-2.1	-4.4	<0.05
Placebo group	15.4	-0.5	-1.1	1.12

GFR: Glomerular filtration rate, MDRD: Modification of diet in renal disease

Table 3: Secondary and tertiary level outcome between the treatment group and placebo group

Parameters	Treatment group	Placebo group	Hazard ratio (CI95%)	p-value
Myocardial Infarction	3	7	4.1	<0.03
Stroke	1	4	3.1	<0.04
Revascularization	5	5	0.0	1.00
Mortality rate	1	3	1.7	1.10
Amputation	1	5	4.7	<0.05
Total	11	24	3.5	<0.05

Analysis of total plasma homocysteine levels: The total plasma homocysteine level in the treatment group at 18 months was decreased to -2.1 µmol L^{-1} at 18 months and -4.4 µmol L^{-1} at 36 months. In the placebo group also the total plasma homocysteine levels were decreased to -0.5 µmol L^{-1} at 18 months and -1.1 µmol L^{-1} at 36 months. The results showed that treatment group subjects showed statistical significant decrease in plasma homocysteine level at 18 months and at 36 months when compared with the baseline and placebo group with p-value of less than 0.05 (Table 2).

Analysis of secondary and tertiary level outcomes: After the 36 months of follow up period, the events such as myocardial infarction, stroke in the treatment group were lower as compared to the placebo group with significant p-value of less than 0.05. However, the rate of mortality between the treatment group and placebo group showed non-significant p-value (Table 3). The end point risk in the treatment group was 12% as compared to placebo group which was 24.2% as shown in (Fig. 1).

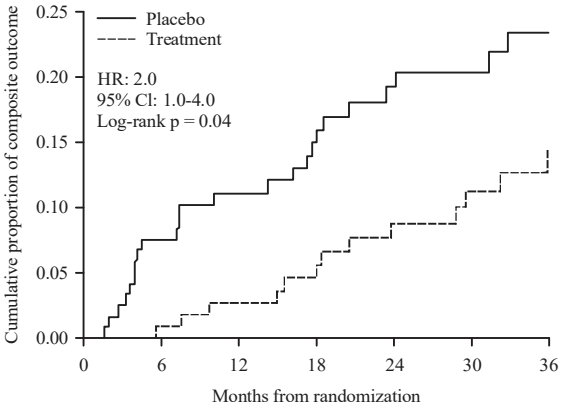

Fig. 1: End point risk assessment between the treatment group and placebo group

DISCUSSION

This randomized trial revealed that the treatment group subjects had higher level of radionuclide, MDRD, GFR levels and reduced urine/albumin ratio at 18 months and 36 months when compared with the baseline level and the placebo group with statistical significant p-value of less than 0.05.

Xiao-Yu Lou *et al.* concluded that breviscapine can reduce cisplatin-induced renal toxicity by inhibiting lipid peroxidation and this method has good clinical feasibility. This means breviscapine combined with cisplatin may be applied to tumor patients to improve the chemotherapy efficiency in the near future[14]. A scientist concluded that the microalbuminuria considered to be the first sign of diabetic nephropathy. They also revealed that breviscapine helps in reducing the urinary protein levels, 24 h urine protein levels and urinary albumin extraction rate which further improves the diabetic nephropathy stage and proved the reno-protective effect[15]. The meta-analysis was done by Liu *et al.*[16] also favors the results of this study and concluded that the BUN and SCR levels were statistically significant lower in the breviscapine treated patients as compared to the control subjects. Zhao[17] concluded that the breviscapine effects urine albumin ratio and improved the quality of life.

The present study evaluated that the treatment group subjects had significantly reduced LDL levels, triglyceride levels, diastolic blood pressure, Insulin levels and BUN. The study was done by Wu *et al.*[18] favored the results of this study and concluded that breviscapine injection can serve as a renal protective effect in patients with hypertensive nephropathy.

The results of this study also showed that the treatment group subjects revealed significantly lower levels of total plasma homocysteine level at 18 months and 36 months as compared to the placebo group. Previous study revealed that the homocysteine levels were considered to be the risk factor for the vascular diseases, so lowering the homocysteine levels definitely improves the quality of life and reduced the complication of diabetic nephropathy[13]. Some of the authors concluded that the high levels of homocysteine mediate cardiovascular problems by its adverse effects on cardiovascular endothelium and smooth muscle cells with resultant alterations in subclinical arterial structure and function[19].

It was also studied that the deregulation of lipid profile causes the progression of diabetic nephropathy and make the situation worsen[20]. Zhi *et al.*[21] also supported that the breviscapine had an excellent effect in lowering the triglyceride level, LDL and increasing the HDL level which further improves the renal function. The results of these studies were also correlated with the results of present study. In the current study, the subjects of the treatment group analyzed the lower rate of adverse effects such as MI and stroke as compared to the placebo group. So, this study stressed that the combination of breviscapine and vitamin B12 improved the GFR levels, decrease in urine albumin ratio, regulate the dyslipidemia, lower the plasma homocysteine level and decrease the adverse effects.

CONCLUSION

This study concluded that the breviscapine improves the diabetic nephropathy sign and symptoms, further improves the quality of life of the subjects. However, the present study explored only the clinical effect of breviscapine with methylcobalamin and further researches are required that will adopt high quality methodology with large samples and conduct long-term follow ups of patients treated with breviscapine in order to investigate its long-term safety.

SIGNIFICANCE STATEMENT

This study discovered that the breviscapin improves the diabetic nephropathy sign and symptoms and further improves the quality of life of the subjects that can be beneficial for patients having the diabetic nephropathy even at the stage III. Breviscapin and vitamin B12 helps in achieving greater increase in GFR, decreased in urine albumin ratio, regulation of dyslipidemia, decrease in plasma homocysteine level and decrease the adverse effects such as MI and stroke. This study will help the researchers to uncover the critical areas of effects of brescia vaccine on the critical stages of diabetic nephropathy that many researchers were not able to explore. Thus a new theory on combination therapy of breviscapin with vitamin B12 may be arrived to provide the patients of diabetic nephropathy with new and safe treatment options.

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