

Original Article

## COMPARISON OF ANGIOTENSIN RECEPTOR BLOCKER ALONE AND IN COMBINATION WITH VITAMIN D ANALOGUE IN REDUCING PROTEINURIA IN DIABETIC NEPHROPATHY PATIENTS

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Received: 25 Jun 2014 Revised and Accepted: 24 Jul 2014

### ABSTRACT

**Objective:** This study was designed to determine the synergistic effect of vitamin D analog added to angiotensin receptor blocker in reducing proteinuria in diabetic nephropathy patients.

**Methods:** A randomized, open label, prospective, parallel assignment, comparative study included 50 patients with diabetic nephropathy who had a spot protein to creatinine ratio (PCR) of 100 - 1000 mg/g creatinine. Patients were randomized to receive treatment with either telmisartan 40 mg or calcitriol 0.25 mcg for a period of 12 weeks. The primary end point was the difference in the spot urinary PCR between the groups at 12 weeks. The secondary endpoints are change in Blood Pressure (BP), serum creatinine and glomerular filtration rate (GFR).

**Results:** The primary end point of the study was reduced by 43.2% with telmisartan and 77.049% with telmisartan and calcitriol treatment (P= 0.0001) during 12 week periods. In the secondary end points, there were no significant differences.

**Conclusion:** Hence, this study concludes that treatment with both calcitriol and telmisartan offers synergistic renoprotection.

**Keywords:** Diabetic nephropathy, Proteinuria, Telmisartan, Calcitriol.

### INTRODUCTION

Diabetes mellitus is increasing in prevalence worldwide and is currently estimated to affect more than 6.5% of the population of the United States [1]. Diabetic nephropathy (DN) is a common presentation of uncontrolled and long standing diabetes. Diabetic kidney disease affects about 15-25% of type 1 diabetes mellitus and 30-40% of type 2 diabetes mellitus. DN is the most common cause of end stage renal disease (ESRD) in the western world [2]. Persistent albuminuria in the range of 30-299 mg/24h or 30-300mg/g creatinine (microalbuminuria) has been shown to be the earliest stage of DN in type1 diabetes and a marker for development of nephropathy in type 2 diabetes. Patients with microalbuminuria who progress to macroalbuminuria (≥ 300 mg/24h or ≥ 300mg/g creatinine) are likely to progress to ESRD over a period of years [3]. In diabetic patients, albuminuria independently predicts renal and cardiovascular outcomes. Several clinical studies have early demonstrated that blockade of the Renin Angiotensin System (RAS) either by an Angiotensin Converting Enzyme Inhibitor (ACEI) (or) Angiotensin Receptor Blocker (ARB) reduce albuminuria retard the progressive loss of renal function and improve survival [4].

The Bergamo Nephrology Diabetes Complication (BENEDICT), Irbesartan in Micro-Albuminuric -2 (IRMA-2) and Action in Diabetes and Vascular Disease: Preterax and Diamicon- MR Controlled Evaluation (ADVANCE) Trials demonstrated that early intervention with a treatment regimen based on an ACEI or ARB in patients with type 2 diabetes and normal albuminuria or elevated albuminuria or overt nephropathy.

Post hoc analysis from the RENAAL trial showed that the initial reduction in albuminuria achieved with an ACEI or ARB predicts a substantial part of its long term renal and cardiovascular protective effect [5]. These data, thus support treatment strategies that focus on reducing albuminuria to achieve long term renal and cardiovascular protection. It is therefore necessary to search for new treatment strategies to reduce the burden of renal and cardiovascular disease in this patient.

1, 25-dihydroxy vitamin D<sub>3</sub> (1, 25 (OH) 2D<sub>3</sub>), the hormonal form of vitamin D, is an important negative regulator of renin biosynthesis. The suppressive activity of 1, 25, (OH) 2D<sub>3</sub> is mediated by the

vitamin D receptor (VDR), a member of the nuclear receptor super family. Vitamin D suppresses renin gene expression by targeting the cAMP Signaling pathway, a major regulatory pathway involved in renin biosynthesis [6]. Although RAS inhibitors, including ACEIs and ARBs, are widely used in the therapy of renal and cardiovascular diseases, the major problem with these drugs is the compensatory renin increase due to the disruption of the feedback inhibition of renin production. The increase in renin activity stimulates the conversion of angiotensin I and ultimately Angiotensin II, which largely limits the efficacy of RAS inhibition.

Combining vitamin D analogs with RAS inhibitors to suppress the reactive renin increase should generate better therapeutic effects. Animal studies proved that the combination of losartan and paricalcitol offers synergistic renoprotection because of more effective inhibition of the RAS within the kidney [7].

This study aimed to prospectively compare the effects of vitamin D analogues (Calcitriol 0.25mcg) added to ARB (Telmisartan 40 mg) with ARB monotherapy in a group of type 2 diabetic nephropathy subjects.

### MATERIALS AND METHODS

#### Setting

From the outpatient department of the Arthur Asirvatham Diabetology Hospital, Madurai, Tamilnadu, we included 50 patients with type 2 diabetic nephropathy.

#### Ethical committee approval

Ethical committee approval was sought from the Institutional Review Board, Arthur Asirvatham Hospital, Madurai, Tamilnadu.

#### Study population

The criteria for inclusion were as follows:

(1) Male or female subjects 35- 80 years old (2) Diagnosis of type II diabetes (3) Hemoglobin A1c concentration (HbA 1c) < 10% (4) Serum creatinine < 3mg/dl (if women) or < 3.2 mg /dl (if men) (5) Glomerular filtration rate (GFR) between 15- 70 ml/min/1.73m<sup>2</sup> (6) Spot urinary protein to creatinine ratio 100 to 1000 mg/g

creatinine(7) Mean systolic and diastolic blood pressure of < 150/100 mm Hg (8) serum calcium level  $\leq$  9.8 mg/dl

Exclusion criteria included (1) Type I diabetes mellitus (2) Pre-menopausal women (3) Known causes of renal dysfunction other than diabetic nephropathy (4) subjects with systolic BP  $\geq$  160 mmHg and or diastolic BP  $\geq$  100 mmHg (5) Known hypersensitivity to Telmisartan or ACE inhibitors or to any component of the formulation (6) Hypercalcemia or vitamin D toxicity (7) Pregnancy and lactation women. A washout period of 2 weeks was given to patients who were on prior antihypertensive therapy.

### Study design

This is a prospective, randomized, open label, single center, parallel assignment, comparative study. After a 2 week washout period, eligible patients were randomized to Telmisartan or Telmisartan and Calcitriol treatment.

### Interventions

Based on the standard dose, patients were randomized using blocked randomization methods and given either of the following preparations

- (1) Telmisartan 40 mg - monotherapy
- (2) Telmisartan 40 mg and Calcitriol 0.25 mcg - combination therapy

Twenty five patients received monotherapy and twenty five patients received combination therapy. Both medications were administered once daily, per orally for a period of 12 weeks.

### Study end points

The primary efficacy endpoint was change from baseline in spot Urinary Protein to Creatinine Ratio (PCR). The secondary end points were change from baseline in systolic and diastolic blood pressure, serum creatinine and Glomerular Filtration Rate using the Modified Diet in Renal Disease (MDRD) equation.

The urine albumin concentration was determined by immunoturbidimetry and serum creatinine was determined by means of the Jaffe reaction (with the use of a Roche kit). The MDRD formula was used to estimate the GFR.

### Statistical analysis

The information collected regarding all the selected cases were recorded in a master chart. Data analysis was done with the help of computer using graph pad Instat DTCG (GPI V3.0). Using these software frequencies, percentages, means, standard deviation and 'p' values were calculated. Mann - Whitney U test was used to test the significances of difference between quantitative variables and Yates test for qualitative variables. A 'p' value less than 0.05 was taken to denote a significant relationship. Efficacy was estimated by measuring the average UPC before and after treatment. Results were expressed as a mean  $\pm$  standard deviation.

## RESULTS

### Characteristics of the patients

A total of 50 patients were randomized, all of whom completed the study. However, 4 patients were not followed up properly. All patients included in the study were mostly men (29 men and 21 women). The mean (SD) age of the patients was  $61 \pm 8.4$  years in the Telmisartan group and  $56.6 \pm 9.7$  years in Telmisartan and Calcitriol group. A total of 43 patients had hypertension, whereas 38 patients had dyslipidemia. Out of 29 male patients, 18 patients are having smoking habit.

Out of the 50 student population, 37 patients were having a family history of diabetes and 13 patients don't have a family history of diabetes. The known duration of diabetes was  $9.07 \pm 6.48$  years in Telmisartan and  $12.18 \pm 7.11$  years in Telmisartan and Calcitriol group. Additional blood pressure, lipid lowering drugs that were taken by the patients in the two treatment groups are shown in Table No: 1.

**Table 1: Baseline characteristics of the randomized populations**

Characteristic	Telmisartan (N=22)	Telmisartan and Calcitriol (N=24)	P Value
Age-yr	61 $\pm$ 8.4	56.6 $\pm$ 7.9	0.1173
Sex-no (%)			
Male	12(54.5)	17(70.83)	0.4023
Female	10(59.0)	7(33.33)	
<b>Family History</b>			
Presence	15(81.81)	18(79.16)	0.853
Absence	7(31.81)	6(25)	
<b>Clinical</b>			
Duration of DM-yr	9.07 $\pm$ 6.48	12.18 $\pm$ 7.11	0.1588
Blood pressure mm Hg			
Systolic	142 $\pm$ 21	133.2 $\pm$ 19.8	0.1370
Diastolic	78 $\pm$ 9.3	78.8 $\pm$ 8.3	0.7816
<b>Medical history-No(%)</b>			
Hypertension	21(95.4)	22(91.66)	0.533
Dyslipidemia	15(68.18)	23(95.83)	0.0167
Neuropathy	9(40.9)	8(33.33)	0.8212
Chronic renal failure	3(13.6)	1(4.16)	0.2713
IHD	2(9.09)	6(25)	0.1612
Atherosclerosis	4(18.18)	8(33.33)	0.4049
Retinopathy	5(22.72)	4(16.67)	0.4412
Urinary PCR ratio (mg/g creatinine)	428.9 $\pm$ 350.3	395.33 $\pm$ 322.97	0.8004
Estimated GFR (ml/min 1.73m <sup>2</sup> )	60.3 $\pm$ 19.5	68.04 $\pm$ 17.56	0.09609
Serum creatinine(mg/dl)	1.23 $\pm$ 0.41	1.25 $\pm$ 0.41	0.3065
Glucose lowering therapy-no(%)	18(81.81)	21(87.5)	0.449
<b>Insulin and Insulin Analogues</b>	15(68.18)	17(70.83)	0.9001
Biguanides			
Sulfonyl urea	12(54.54)	15(62.5)	0.8044
Thiazolidine diones	7(31.81)	6(25)	0.853
<b>Lipid lowering drugs-no(%)</b>			
Statin	21(95.45)	21(87.5)	0.338
Fibrates	5(22.72)	8(33.5)	0.6382

- Plus-minus values are mean ± SD. To convert the values for serum creatinine to micromoles per liter, multiply by 88.4.
- Data are for 12 men in Telmisartan group & 17 men in Telmisartan and Calcitriol group. 13 women in the Telmisartan group and 8 women in the Telmisartan and Calcitriol group.
- The glomerular filtration rate was calculated with the use of the modification of diet in renal disease (MDRD) formula.
- IHD – Ischemic Heart Disease.

This prospective, randomized, open label, single centre, parallel assignment study compared Telmisartan (40 mg) with Telmisartan (40 mg) and Calcitriol (0.25mcg) in type 2 diabetic nephropathy patients. It was conducted at Arthur Asirvatham Hospital, Madurai, Tamilnadu, with 60 outpatients meeting inclusion criteria and 50 patients randomized to treatment. Out of 50 patients, only 4 patients dropped out throughout the study. Remaining 46 patients were followed up properly. 24 and 22 patients were in Telmisartan and Telmisartan with Calcitriol treatment group respectively.

**Primary end point**

**Protein to creatinine ratio**

By the end of the study period, treatment with Telmisartan ( 40 mg orally once daily for 12 weeks ) and Calcitriol ( 0.25 mcg orally once daily for 12 weeks ) had reduced the mean urinary protein to creatinine ratio by 43.2%, as compared with Telmisartan monotherapy (40 mg orally once daily for 12 weeks ) by 77.045 ( p = 0.0001)

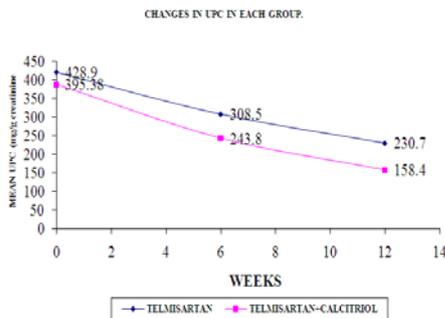


Fig. 1: changes in UPC in each group. The values are representing mean ± s.d.s at each time point

**Secondary end points**

**Serum creatinine**

In the Telmisartan group, the geometric mean value of Serum creatinine was reduced from 1.23 mg/dl to 1.01 mg/dl. In the Telmisartan with Calcitriol group, the corresponding values for baseline and end of treatment were 1.25 mg/dl and 0.96 mg/dl. This translated into a 15.62% reduction with Telmisartan Vs 13.53% with Telmisartan and Calcitriol treatment. There were no significant differences between the two treatment groups.

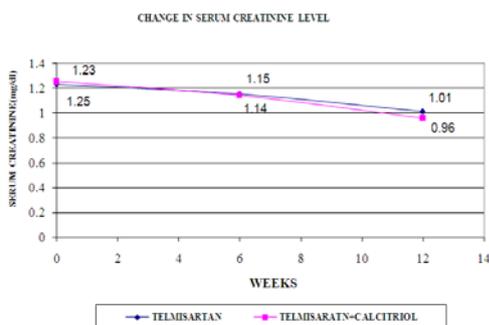


Fig. 2: changes from baseline in the serum creatinine according to the study group.

**GFR rate**

The mean rate of decline in the estimated glomerular filtration rate during the 12 week study period was 7.2 ml/min/1.73m<sup>2</sup> in the Telmisartan treatment group and 4.9 ml/min/1.7m<sup>2</sup> in the Telmisartan and Calcitriol group. It didn't reveal any significant differences (P=0.5526)

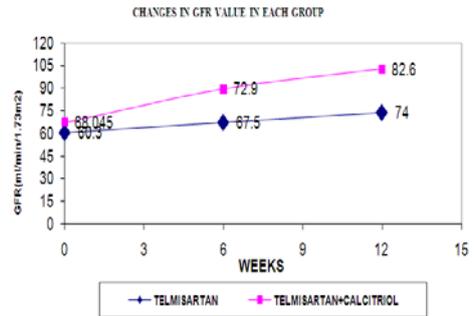


Fig. 3: Relative change from baseline in the serum creatinine after treatment with Telmisartan as compared with Telmisartan and Calcitriol in diabetic nephropathy patients

**Blood Pressure**

Both groups had significant reductions in systolic pressure at trial end, with no significant differences between groups (6.6% in telmisartan group and 2.0% in telmisartan and calcitriol group) (p= 0.28). Diastolic pressure also failed to show consistent difference throughout the study. The reduction of the mean value of diastolic B.P from baseline to 12 week study period was 78 to 77.5 mmHg in the Telmisartan group and 78.8 to 78.6 mmHg in Telmisartan and Calcitriol group. No significant differences have seen between the two treatment groups. (p = 0.88).

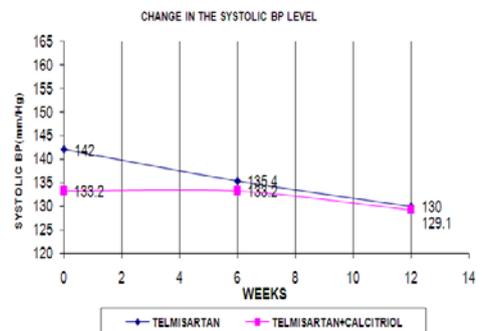


Fig. 4: Changes in the systolic blood pressure in each group. The values represent mean ± s.d.s at each time point.

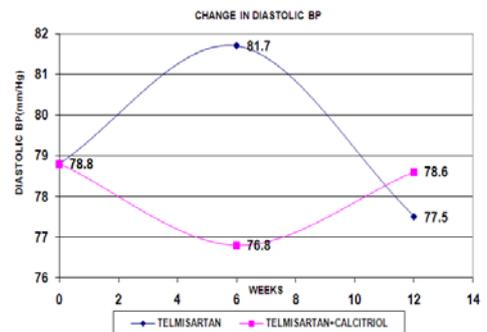


Fig. 5: Changes in the diastolic blood pressure in each group. The values represent mean ± s.d.s at each time point.

## DISCUSSION

The results of this open label, randomized, single center study suggests that in patients with hypertension and diabetic nephropathy, Telmisartan with Calcitriol (vitamin D analog) combination-based regimen is superior to Telmisartan-based regimen for reducing proteinuria. Our study is the first, to our knowledge, to investigate the synergistic effect of Calcitriol with Telmisartan in type 2 diabetes with nephropathy patients. The result suggests that the reduction in proteinuria and blood pressure is primarily caused by interference in the renin-angiotensin system. The reduction of proteinuria by dual blockade of the Renin-Angiotensin System (RAS) was independent of changes in blood pressure, which was only modestly and insignificantly reduced. This change in proteinuria was based on a change in spot urine protein:creatinine ratio. This method overcomes the drawbacks of 24-h urine collection; thus, it is a recommended method for clinical trials. The declining of renal function is strongly associated with lower serum level of 1, 25 hydroxy vitamin D [8]. Possible explanations for this high prevalence of nutritional vitamin D deficiency in patients have reduced sunlight exposure (*e.g.*, in the elderly, the sick, dark-skinned people, those who wear a veil for cultural reasons), losses of vitamin D-binding protein in proteinuric states. The results of a prospective clinical trial to evaluate the effects of short-term (1-month) treatment with activated vitamin D (Paricalcitol) on blood pressure, bio measures of inflammation, endothelial function, and measures of urinary protein excretion. Paricalcitol strongly reduces urinary protein excretion and also on C-reactive protein levels [9]. But there is no apparent effect on blood pressure, measured either in the office or with 24-hour ambulatory blood pressure monitoring, nor was there an effect on renal hemodynamics, vascular function, or Para Thyroid Hormone (PTH) levels. In this study, the benefit of Calcitriol appears to be independent of the systemic blood pressure; systolic and diastolic blood pressures during the study were only marginally lower in the Telmisartan with Calcitriol combination group than in the Telmisartan group. This change may be due to some antihypertensive agents that were continued into the randomized phase included calcium channel blockers, thiazide diuretics, and  $\beta$ -blockers. In clinical studies of vitamin D supplementation they have noted that paricalcitol reduced proteinuria in patients with Chronic Kidney Disease (CKD) [10]. More recently Calcitriol had modest antiproteinuric effects in patients with nephropathy and persistent proteinuria despite renin-angiotensin system blockade [11]. Some other factors that affect the results are diet control (increase intake of protein), patient non compliance (improperly taking medication), psychological factor, and lifestyle of the patients. In this study, the proteinuria reduction observed with Telmisartan and Calcitriol combination regimen was 39.8% and 41.2% versus 43.3%, and 59.9% in Telmisartan regimen during the second and third visit respectively. In both the treatment groups, the greatest reduction in proteinuria was apparent at 12<sup>th</sup> week measurements. Further analysis revealed that of patients who were on ACEI/ARB therapy and had proteinuria at baseline, 52% (22/42) of paricalcitol patients versus 27% (11/41) of placebo patients had decreased proteinuria ( $p=0.025$ ). There was not statistically significant difference between paricalcitol and placebo groups in mean eGFR change from baseline to the final visit ( $-2.50 \pm 0.54$  Vs  $-3.0 \pm 0.53$  ml/min/1.73m<sup>2</sup>, respectively,  $p=0.51$ ). Blood pressure at baseline, week 7, week 15, and the final visit, had no statistically significant difference between the two groups for the mean change of systolic blood pressure/diastolic blood pressure at each visit and from baseline to the final visit [12]. Even though Calcitriol appeared to have a beneficial effect on reducing proteinuria, the effect on serum creatinine, GFR, systolic and diastolic BP level was not always statistically significant. In this study, the mean eGFR value of Telmisartan group and Telmisartan and Calcitriol combination group was not statistically significant during the second ( $P=0.4846$ ) and third visit ( $P=0.5536$ ). In this study, the estimated glomerular filtration rate was nearly identical in the two groups at baseline, whereas the decline in the glomerular filtration rate tended to be smaller among the patients who were treated with Telmisartan and Calcitriol combination for 12 weeks than among the patients who were given Telmisartan.

Long term studies (more than 2 years duration) must be conducted to elucidate whether the beneficial effect on the kidney that is seen in the short term is sustained.

Preventing or delaying the development of diabetic nephropathy is a major goal of treatment. Our findings indicate that this goal can be achieved if high-risk patients are treated with appropriate renoprotective therapy with Telmisartan and Calcitriol combination therapy. Patients with diabetic kidney disease, routine screening of urine for microalbuminuria should be performed in all patients. Unfortunately, patients at high risk for diabetic nephropathy are rarely identified early. It must be emphasized that improvement in glycemic control slows the increase in the level of albuminuria and postpones the occurrence of overt diabetic nephropathy in patients with type 2 diabetes.

## CONCLUSION

In conclusion, our short term study supports the concept that treatment with both Calcitriol and Telmisartan offers synergistic renoprotection, but not obtainable with Telmisartan monotherapy in type 2 diabetic patients with nephropathy.

## CONFLICT OF INTERESTS

Declared None

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