Original Article

A Study of Effect of Angiotensin Converting Enzyme Inhibitor (Ramipril) Therapy on Renal Function and Proteinuria in Type-2 Diabetic Nephropathy Patients

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Abstract
Background: *Diabetic nephropathy characterized by persistent albuminuria is the single leading cause of end-stage renal disease. Renin angiotensin system (RAS) is considered to be involved in most of the pathological processes that result in diabetic nephropathy. The progression of diabetic nephropathy can be retarded by ACE inhibitors (ACEIs) in patients with type 2 diabetes. The aim of our study was to find out the antiproteinuric and renoprotective effect of Ramipril, an angiotensin-converting enzyme (ACE) inhibitor, in diabetic nephropathy patients.*
Materials and Methods: *The study was conducted on 63 patients of diabetic nephropathy of type 2 diabetes mellitus aged between 31-64 years, selected from indoor ward and subsequently followed up as outdoor patients of medicine department of Narayan Medical College and Hospital, Sasaram, Bihar. Treatment with Ramipril (ACEI) was initiated after proper control of blood pressure and plasma glucose. Before treatment and after 2 months of continuous therapy with Ramipril, proteinuria and GFR estimation by creatinine clearance method were assessed.*
Results: *Ramipril treatment improved renal function. After proper control of blood pressure and glycemia, overall GFR improved from 52.26±9.12 to 60.26±13.76 which further improved to 70.26±15.38 ml/min after ACE-inhibitor therapy. Overall proteinuria which was 1898.53±1348.80 mg/24hr before control of BP and glycemia reduced to 1614.26±1163.37 mg/24 hr after control of BP and glycemia and further decreased followed ACE-inhibitor therapy to 1373.26±1289.16 mg/24hr.*
Conclusions: *It was observed that proper control of glycemia and BP may lead to substantial improvement in GFR as well as proteinuria in type 2 diabetic patients with diabetic nephropathy. ACE-inhibitor institution following proper control of BP and glycemia may further improve the GFR and reduce proteinuria.*
Keywords: *Angiotensin-converting enzyme inhibitors, diabetic nephropathy, type 2 diabetes mellitus.*

Introduction

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) worldwide, and it is estimated that ~20% of type 2 diabetic patients reach ESRD during their lifetime.[1] In India, CURES study reported a prevalence of 2.2% for overt diabetic nephropathy and 26.9% for microalbuminuria.[2] These patients are at an increased risk for premature death, cardiovascular disease, and other severe illnesses that result in frequent hospitalizations and increased health-care utilization. Although much progress has been made in slowing the progression of diabetic nephropathy, renal dysfunction and the development of end-stage renal disease remain major concerns in diabetes.[3] Diabetic nephropathy being an inflammatory condition, Angiotensin Ⅱ levels have been found to be elevated. [4]This rise activates immune cells and causes production of chemokines[5] leading to further renal damage. Dysregulation of the renin-angiotensin-aldosterone system (RAAS) results in progressive renal damage and hence RAAS blockade is the cornerstone of treatment of diabetic nephropathy, with proven efficacy in many arenas[6]

Kidney disease in diabetic patients is clinically characterized by increasing rates of urinary albumin excretion, starting from normoalbuminuria, which progresses to microalbuminuria, macroalbuminuria, and eventually to ESRD.[7] Microalbuminuria is the earliest clinically detectable stage of diabetic kidney disease at which appropriate interventions can retard, or reverse, the progress of the disease.[8] ACE inhibitors competitively block RAAS system, decrease glomerular capillary pressure and slow progression from microalbuminuria to gross proteinuria.[9]. Recently, a number of studies have also indicated ACE inhibitors also decrease levels of advanced glycation endproducts (AGE)[10], a major factor in development of diabetic nephropathy.[11] Hence ACE inhibitors have been established to be first-line drugs in preventing the development and retarding the progress of diabetic nephropathy.[12]

On the background of these facts, we planned to study antiproteinuric and renoprotective effect of Ramipril, an angiotensin-converting enzyme (ACE) inhibitor, in diabetic nephropathy patients.

Material & Methods

Approval from the Institutional Ethical committee was taken. The present prospective study was conducted on 63 patients of diabetic nephropathy of type 2 diabetes mellitus aged between 31-64 years, selected from indoor ward and subsequently followed up as outdoor patients of medicine department of Narayan Medical College and Hospital, Sasaram, Bihar.

Subjects: A total of 63 subjects were selected for this study in between Jan 2017 to Dec 2017 out of which finally 30 subjects were followed up with ACE inhibitor (Ramipril) therapy after proper glycemic and satisfactory BP control for 2 months.

Inclusion Criteria

The patients included in the study were subjects with type 2 diabetes mellitus (criteria of diabetes mellitus based on WHO guideline) [13] and who had persistent proteinuria (≥300mg total protein /24hr, measured on two occasions with a gap of one to three months duration, [14]excluding UTI) were selected in this study.

Exclusion Criteria

Patients of type-1 diabetes mellitus, critically ill patients requiring parenteral feeds or IV antibiotics, known kidney disease, chronic kidney disease on dialysis, coronary artery disease, urinary tract infection, and pregnant and lactating women were excluded from the study. All the selected patients were admitted in the ward and a detailed, careful history and thorough physical examination were done. All patients were subjected to X-ray chest, USG abdomen for kidney size, and 2D- Echocardiography.

Biochemical Examination: Plasma sugar- fasting and post-prandial, serum urea, serum creatinine, 24-hr urinary protein& creatinine, serum electrolytes, lipid profile were analyzed in an

automated analyzer. Calculation of GFR was done by creatinine clearance method using the formula

$$C_{cr} = U_{cr} \times V / P_{cr}$$

where U_{cr}= urinary creatinine (mg %), P_{cr}=plasma creatinine concentration mg%, V = Volume of urine per min.

All the patients were discharged after proper glycemic and BP control and were strictly followed up for BP and glycemic control. After 3 months those patients who had good glycemic and BP control were investigated again for serum urea, serum creatinine, 24-hr urinary protein and creatinine and urine culture & sensitivity; and were further followed up with addition of ACE-inhibitor. The ACE inhibitor of Choice was Ramipril, titratable to a maximum dose as the drug was cheaper than other ACE inhibitors and no major recent adverse event profile with good patient counselling and after detailed explanation.

The oral dose of Ramipril ranges from 1.25mg to 20mg daily (single or divided doses). After 2 months of Ramipril therapy, similar biochemical analysis was repeated.

Statistical Analysis: Statistical analysis was performed using MSTAT software. The results were presented as mean ± standard deviation (SD) and percentage. A p value <0.05 was considered statistically significant.

Results

63 patients were included in the study. 54(85.7%) were males and 9(14.3%) were females. Mean age of study population at the time of presentation was 53.33±6.66 years and mean age at the time of detection of diabetes was 43.30±4.8 years. Mean duration of diabetes at the time of presentation was 9.46 ±7.8 years.

Table 1: Correlation between Duration of diabetes and GFR

DURATION (YRS)	GFR (ml/min)		
	>70	30-70	<30
< 5	98.17±15.77 MD=2±0.89 N=6	48.4±9.56 MD=2.4±0.84 N=10	19.5±0.70 MD=2 N=2
5-10	90 MD=6.5±0.7 N=2	43.92±7.18 MD=7.6±1.24 N=13	19 MD=9 N=1
>10	72 MD=14±1.4 N=2	39±5.8 MD=14.8±1.9 N=17	21.9±2.7 MD=17.6±1.95 N=10

MD= Mean Duration, N= Number of patients.

Amongst the patients with duration less than 5 years, 10 cases had GFR in the range of 30-70 (48.4±9.65) ml/min and 6 patients had >70 (98.17±15.77) ml/min.GFR value was decreased with longer duration (43.92±7.18) ml/min with mean duration of (7.38±1.04) yrs. GFR was further decreased to 39±5.8 in those who had diabetic duration of greater than 10 yrs (mean duration 14.8±1.9yrs). A few patients with duration less than 10 yrs had GFR quite less (<30ml/min). (Table-1)

Table 2: Correlation between Duration of diabetes and Proteinuria

DURATION (YRS)	Proteinuria(mg/24 hr)		
	<1000	1000-3000	>3000
< 5	361.3±78.49 MD=2.12±0.83 N=8	1665±481 MD=2.4±0.89 N=5	5733±1090.85 MD=2.2±0.83 N=5
5-10	542.5±112.42 MD=6 N=2	1887±635.2 MD=7.62±1.06 N=8	4086±1167.84 MD=7.5±1.04 N=6
>10	320 MD=13 N=1	1897±502.7 MD=15.16±2.4 N=6	4929.72±779.66 MD=16±2.4 N=22

MD= Mean Duration, N= Number of patients.

Almost half of patients (44.4%) of duration less than 5 yrs had proteinuria less than 1000mg/24hr.while in second group (duration 5-10yrs), majority of patients (87.5%) had proteinuria more than 1000mg/24hr while 37.5%

patients of this group had proteinuria more than 3000mg/24 hr. In third group duration of diabetes >10 yrs, most of the patients (75.86%) had proteinuria more than 3000 mg/24 hr. (Table-2)

Table 3: Correlation between GFR and Proteinuria

GFR (ml/min)	Proteinuria (mg/24 hr)		
	<1000	**1000-3000**	**>3000**
>70	363.57±114.7 MG=94.57±17.42 N=7	1317.5±215.6 MG=81±12.7 N=2	6588 MG=89 N=1
30-70	437.5±83.55 MG=53.75±8.01 N=4	1828.46±543.2 MG=46.94±6.69 N=17	4511.26±1050.43 MG=36.73±3.63 N=19
< 30	- N=0	- N=0	4906.3±691.23 MG=21.30±2.62 N=13

MG= Mean GFR, N= Number of patients

In first group (GFR >70 ml/min) only 10% had proteinuria >3000 mg/hr while 70% cases had proteinuria <1000 mg/24 hr. In second group (GFR 30-70 ml/min) 47.5% had proteinuria >3000

mg/24 hr, 42.5% in the range of 1000- 3000 mg/24 hr while 10% had proteinuria <1000 mg/24 hr. In third group (GFR <30 ml/min) all had proteinuria >3000 mg/24 hr. (Table-3)

Table 4: Correlation between Systolic Blood Pressure (SBP) and GFR

SBP (mm of Hg)	GFR (ml/min)		
	>70	**30-70**	**<30**
< 140	92±17.08 MS=118.66±6.11 N=3	45.06±9.46 MS=122.26±7.95 N=15	20.75±3.09 MS=120±1.63 N=4
140-159	96.5±20.02 MS=145±2.58 N=4	43.41±7.73 MS=148.16±2.75 N=12	20.82.86 MS=152.4±1.67 N=5
160-179	89.5±0.70 MS=166±5.65 N=2	41.44±5.02 MS=164.66±3.87 N=10	22.5±3.53 MS=169±1.41 N=2
>180	72 MS=186 N=1	47.66±6.02 MS=188±8 N=3	22.5±0.70 MS=191±12.72 N=2

MS= Mean SBP, N= Number of patients.

Two third of patient with SBP less than 140 mm Hg had GFR 45.06 ±9.46 ml/min, 18.18% had <30 ml/min while 13.6% had >70 ml/min. Most of the patient (57.14%) with SBP range of 140-159 mm Hg had 43.41±7.73 ml/min GFR. In third

group with SBP range of 160-179 mm Hg most patients had mean GFR 41.44±5.02 ml/min. Only 6 patients had SBP >180 mm Hg, among which 3 had GFR 30-70ml/min, 2 had < 30 ml/min and 1 had GFR >70 ml/min. (Table-4)

Table 5: Correlation between Systolic Blood Pressure (SBP) and Proteinuria

SBP (mm of Hg)	Proteinuria (mg/24 hr)		
	<1000	**1000-3000**	**>3000**
< 140	434.4±138.6 MS=120±6.32 N=5	1818.25±614.92 MS=126.75±7.4 N=8	4565.33±1032.78 MS=117.33±3.16 N=9
140-159	335±39.87 MS=146.5±1.91 N=4	1719.2±409.9 MS=147.2±4.38 N=5	4896.91±912.7 MS=149.83±3.12 N=12
160-179	N=0	2069.25±607.19 MS=161±1.15 N=4	5148.3±1156.36 MS=167.2±2.85 N=10
>180	391.5±101.11 MS=187±1.41 N=2	1651.5±645.5 MS=188±11.31 N=2	5150±565.68 MS=191±2.72 N=2

MS= Mean SBP, N= Number of patients

In patients with SBP<140 mm of Hg, 5 had proteinuria <1000 mg/24 hr, 8 in the range of 1000-3000 mg/24 hr, while 9 cases had >3000 mg/24 hr. In second group (SBP 140-159 mm of Hg) and third group (SBP 160-179 mm of Hg),

majority of patient had proteinuria >3000 mg/24 hr. Only 6 cases were recorded with SBP> 180 mm of Hg, 2 in the range of <1000 mg/24 hr, 2 case had 1000-3000 mg/24 hr proteinuria and 2 case had >3000 mg/24 hr proteinuria (Table-5)

Table 6: Effect of ACE- inhibitor therapy after proper BP and glycemic control on renal function and Proteinuria

GFR (ml/min)	Before proper control of BP & glycemia	After proper control of BP &glycemia	
		Before Ramipril therapy	After Ramipril therapy
>70	81±12.72 N=4	83.66±10.01 N=6	80.22±6.93 N=18
30-70	47.84±6.68 N=26	54.41±8.89 N=24	55.33±9.99 N=12
<30	- N=0	- N=0	- N=0
Overall N=30	52.26±9.12	60.26±13.76	70.26±15.38
Proteinuria (mg/24 hr)			
<1000	542.33±79.5 N=6	661±285.80 N=10	685±252.91 N=18
1000-3000	1573.88±514.50 N=18	1581.12±554.70 N=16	1786.8±605.7 N=10
>3000	4228.66±1120.43 N=6	4130±664.68 N=4	5500 N=2
Overall N=30	1898.53±1348.80	1614.26±1163.37	1373.26±1289.16

There was overall increment in GFR. Before control of glycemia and BP mean GFR was 52.26± 9.12 ml/min. After proper control of glycemia and BP the overall GFR increased to 60.26±13.76 ml/min which further improved to70.26±15.38 ml/min after Ramipril therapy. Similarly overall improvement in proteinuria was observed. Before control of BP and glycemia mean proteinuria was 1898.53±1348.80 mg/24 hr which was decreased after proper control of BP and glycemia itself 1614.26±1163.37 mg/24 hr and further decreased after ACE-I therapy to mean value of 1373.26±1289.16. (Table 6)

Discussion

Nephropathy is a major cause of illness and death in diabetes. Proteinuria is a key feature of diabetic nephropathy and a strong predictor of speed of progression towards end stage renal failure. Manoeuvres that lessen proteinuria have significant renoprotective effect. In the present study, about 76% patients presented with a clinical evidence of proteinuria were more than 50 years of age group (mean age was 53.33±6.66 years), whereas more than 74% patients were detected as

diabetic at the age of more than 40 years (mean age at the time of detection of diabetes 43.30±4.8 years). Previous studies and this study, have found that awareness regarding diabetes mellitus and its complications is low even among individuals who have the disease leading to late detection of cases. [15] In many parts of the country, particularly the prevalence of undiagnosed diabetes mellitus is high, with nearly three undiagnosed individuals for every known case.[16]

Mean duration of diabetes at the time of presentation to this hospital with a diagnosis of diabetic nephropathy was 9.46±7.8 years. About 50% of patients were suffering from diabetes from >10 years. This difference is almost two third from >5 years, probably because of overall late detection due to unawareness of early diabetic symptoms, frequently occurring in villagers who are mostly uneducated and belong to low socio economic status. This observation is very similar to the result obtained in the UKPDS 64 study in which mean duration of diabetes at the time of presentation with a diagnosis of diabetes nephropathy was around10 years.[17]

During examination diabetic retinopathy was the commonest finding amongst all diabetic complication seen in >85%, followed by peripheral neuropathy (69.84%) and coronary artery disease (41.26%) This observation is very similar to the result obtained in the study done by Chandy A et al in Indian population. [18]

There was inverse correlation between duration of diabetes and GFR in our study. Those who were suffering from diabetes for more than 10 years (mean 14.8±1.9), renal impairment was quite advance (mean GFR39±5.8) and one third of them had GFR <30 ml/min. Like GFR, proteinuria was showing an obvious positive correlation with duration of diabetes. 44.44% of patients with short duration (<5years) had mild proteinuria (<1000mg/24 hr) while almost three fourth of those with longer duration (>10years) had nephritic range proteinuria (>3000mg/24 hr). Findings are consistent with previous study in South Indian Population. [2]

The current study showed that proteinuria was inversely correlated with GFR. As the renal impairment progresses, proteinuria tends to rise. In the subjects, those who had severe renal impairment (GFR<30ml/min), all had nephrotic range proteinuria. Our finding is well supported by study of Rossing K et al.[19]

In this study, we observed there was overall deterioration in GFR with progression of SBP. Similarly proteinuria tends to increase when SBP was high. Among those patients having SBP within normal range (SBP <140 mm Hg) 59% had proteinuria below nephrotic range but amongst those who were hypertensive (SBP>140 mm Hg), 60% had nephrotic range proteinuria. In their study, Knowler W G et al found that in type 2 diabetes mellitus patients the risk of developing clinical proteinuria is increased more than twofold in patients with blood pressure >165/95 mm Hg compared to those with lower blood pressure after adjusting for age, sex and duration of diabetes, which supports our finding in the present study.[20]

We followed up 30 patients with ACE-inhibitor (Ramipril) therapy after proper glycemic and BP control for 2 months. We noted, there was overall improvement in GFR and reduction in proteinuria merely by controlling BP and glycemia and further improved after introduction of ACE-inhibitor therapy.

After proper control of glycemic and BP overall GFR improved from (52.26 ± 9.12 to 60.26 ± 13.76) which further improved to70.26 ± 15.38 ml/ min after Ramipril therapy. Similar favourable effect was observed regarding proteinuria. Overall proteinuria reduced from (1898.53 ± 1348.80) to (1614.26 ± 1163.37) after proper control of BP and glycemia, and further decreased followed ACE-inhibitor therapy to (1373.26 ± 1289.16) mg/24 hr. These findings are well comparable to numerous related studies concerning ACE-inhibitor in type 2 diabetes mellitus patients. [21]In a meta-regression analysis of 100 studies, Kasiske et al found that only ACE inhibitors were able to reduce the level of proteinuria and slow the rate of decline in renal function regardless of changes in blood pressure, which further support our result.[22]

Conclusion

In the present study it was observed that there was inverse correlation between duration of diabetes with GFR and positive correlation with proteinuria. There was constant tendency of deterioration in renal function with progression of SBP. Overall, GFR has inverse correlation with proteinuria, as GFR decreases proteinuria aggravates. In the follow up study based on 30 patients, it was observed that proper control of glycemia and BP may lead to substantial improvement in GFR as well as proteinuria in type 2 diabetic patients with diabetic nephropathy. ACE-inhibitor institution following proper control of BP and glycemia may further improve the GFR and reduce proteinuria.

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