

STUDY TO COMPARE THE THERAPEUTIC EFFICACY OF N-ACETYL CYSTEINE AND COMBINATION OF N-ACETYL CYSTEINE AND TAURINE IN PROTEINURIA IN DIABETIC NEPHROPATHY

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Received: 26 September 2016, Revised and Accepted: 01 November 2016

ABSTRACT

Objective: Proteinuria is an established risk factor for progressive damage of kidney. This proteinuria can be effectively controlled by drugs that interrupt the renin-angiotensin-aldosterone system. However, the efficacy of N-acetyl cysteine (NAC) and taurine in prevention of diabetic nephropathy (DN) is uncertain. The role of NAC and taurine in reducing of proteinuria in patients with DN was studied.

Methods: The present study was undertaken in the Postgraduate Department of Medicine of S.N. Medical College, Agra. A total of 60 patients of diabetes mellitus with evidence of nephropathy were included in the study. Twenty patients were given placebo medication (Group P), 20 patients received NAC (Group A), and remaining 20 received a combination of NAC and taurine (Group B). Each group was further subdivided according to the urinary albumin excretion in 24 hrs. Subgroup 1 - Urinary albumin excretion between 30 and 300 mg/24 hrs. Subgroup 2 - Urinary albumin excretion >300 mg/24 hrs. The statistical analysis used Student's *t*-test.

Results: On comparison, in both microalbuminuria and macroalbuminuria patients divided into three groups; the difference in magnitude of increase in proteinuria (after NAC, NAC with taurine, and placebo administration) over 24 weeks of the study period was statistically insignificant.

Conclusions: In this study, there was no significant difference of proteinuria before and after NAC administration, and also in our study, there was no significant difference of proteinuria before and after taurine administration.

Keywords: Albuminuria, Diabetic nephropathy, N-acetyl cysteine, Taurine.

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INTRODUCTION

Diabetic nephropathy (DN) is the leading cause of mortality and morbidity in diabetic patients. It is also the leading cause of end-stage renal disease (ESRD). The proportion of diabetes-related ESRD among all cases of ESRD is reported to be about 25-55%. The pathological abnormality found in DN includes mesangial expansion, basement membrane thickening, glomerulo- and arteriosclerosis, changes in endothelial and tubular cells, podocyte abnormalities, and interstitial inflammation [1-3]. However, the excessive deposition of extracellular matrix (ECM) proteins in mesangium and basement membrane of glomerulus are the earliest morphological changes and typical hallmarks of DN [4,5].

The earliest clinical evidence of DN is microalbuminuria [6]. Glomerular hemodynamic changes such as hyperperfusion and hyperfiltration cause leakage of albumin from glomerulus into Bowman's space [7]. There are many factors including nitric oxide (NO), insulin-like growth factors, angiotensin II, vascular endothelial growth factor, transforming growth factor β_1 (TGF β_1), cytokines, and reactive oxygen species (ROS) have been implicated in pathogenesis of DN. Hyperglycemia induces endothelial apoptotic cell death and vascular endothelial dysfunction (VED) [8]. More precisely, hyperglycemia leads to production of ROS, which in turn causes downregulation of expression of NO synthase [8]. VED results in decreased production of NO and increased production of ROS. It is well known that blockage of renin angiotensin aldosterone system prevents microalbuminuria progression and ultimately chronic renal damage [9].

Oxidative stress is a well-known phenomenon in pathogenesis of DN. It has been seen that antioxidants such as vitamin E, vitamin C, and statins reduce proteinuria in diabetic patients. Human studies on N-acetyl

cysteine (NAC) as an antioxidative agent are limited; the majority of studies are on animal models. NAC in experimental models has revealed a reduction in ischemia in acute kidney damages, improved glomerular filtration rate, and shortened recovery periods [10-13]. Studies of human beings on the role of NAC in the treatment of DN and proteinuria are not frequent and findings are controversial [14-17].

Taurine (2-aminoethanesulfonic acid) is considered an endogenous antioxidant. Recent studies have shown that taurine protects renal tissues against damage from oxidative stress [18-21]. To further investigate, we studied the therapeutic efficacy of NAC and taurine in proteinuria in DN.

METHODS

The present study was undertaken in the Postgraduate Department of Medicine of S.N. Medical College, Agra. A total of 60 patients of diabetes mellitus with evidence of nephropathy were included in the study. Patients with known hypersensitivity to NAC/taurine, with chronic liver disease, patients with non-diabetic nephrotic cause of proteinuria, patients with acid peptic disease, pregnant and lactating women, patients with uncontrolled hypertension, patients with coronary artery disease, patients who have had cerebrovascular accident within last 1 year, patients with evidence of heart failure, and patients taking angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), and calcium channel blockers were excluded from the study. Ethical clearance was taken from Ethical Committee. After taking informed consent, three groups were made according to medication used. Twenty patients were given placebo medication (Group P), 20 patients received NAC (Group A), and remaining 20 received a combination of NAC and taurine (Group B).

- Group P - Received placebo
- Group A - Received NAC
- Group B - Received combination of NAC and taurine.

Each group was further subdivided according to the urinary albumin excretion in 24 hrs.

- Subgroup 1 - Urinary albumin excretion between 30 and 300 mg/24 hrs
- Subgroup 2 - Urinary albumin excretion >300 mg/24 hrs

The enrolled patients were subjected to detailed clinical examination. Cases were subjected to the following investigations:

Hemogram, blood sugar (fasting and postprandial), kidney function test, liver function test, urine analysis, 24 hrs urine protein, fundus examination, ultrasonography for kidney size, and echogenicity. Patients were followed up at 24 weeks. Tight glycemic control was maintained in all groups, and drug compliance was assured.

Table 1: Age distribution of cases

Age range (in years)	Group P	Group A	Group B
25-35	2	2	0
36-45	7	8	8
46-55	8	10	12
56-65	3	0	0
Total	20	20	20

Table 2: Sex distribution of cases

Sex	N (%)		
	Group P	Group A	Group B
Male	12 (60.00)	15 (75)	14 (70)
Female	8 (40.00)	5 (25)	6 (30)
Total	14 (100)	26 (100)	20 (100)

Table 3: Microalbuminuria at baseline and 24 weeks (Subgroup 1 in all these groups)

Group	Subgroup	Number	24 hrs proteinuria baseline (mg/24 hrs)	24 hrs proteinuria at 24 weeks (mg/24 hrs)	% change over baseline
P	1	11	222.25±66.25	251.5±55.58	13.16
A	1	8	209.86±64.32	229.29±60.52	9.26
B	1	12	225.67±49.60	244.33±53.61	8.27
Subgroups 1		24 hrs proteinuria baseline (mg/24 hrs)		24 hrs proteinuria at 24 weeks (mg/24 hrs)	
		t-value	p value	t-value	p value
Groups P and A		0.4093	0.4093 ^{NS}	0.8282	0.421 ^{NS}
Groups P and B		-0.1392	0.8908 ^{NS}	0.3275	0.7466 ^{NS}
Groups A and B		-0.5883	0.5669 ^{NS}	-0.5695	0.5781 ^{NS}

NS: The two samples are not significantly different

Table 4: Overt albuminuria/24 hrs at baseline and at 24 weeks (Subgroup in all these groups)

Group	Subgroup	N	Baseline proteinuria (mg/24 hrs)	Proteinuria at 24 weeks (mg/24 hrs)	% Change over baseline
P	2	9	609.67±147.03	657.33±154.20	7.82
A	2	12	628.67±200.83	671.83±222.55	6.87
B	2	8	642.75±205.53	685.00±218.57	6.57
Subgroups 2		Proteinuria baseline (mg/24 hrs)		Proteinuria at 24 weeks (mg/24 hrs)	
		t-value	p value	t-value	p value
Group P and A		-0.2351	0.8166 ^{NS}	-1762	0.862 ^{NS}
Group P and B		-0.3774	0.7122 ^{NS}	-0.2981	0.7705 ^{NS}
Group A and B		-0.1515	0.8816 ^{NS}	-0.1311	0.8974 ^{NS}

NS: The two samples are not significantly different

RESULTS

A maximum number of cases were in the age group of 46-55 years (Table 1).

There is male preponderance in all the three groups. This can be explained by as males are attending the outpatient department more than females in numbers (Table 2).

In patients with microalbuminuria and receiving placebo drug (Group P1), mean baseline value of microalbuminuria was 222.25±66.25 mg/24 hrs, and at 24 weeks, it was 251.50±55.58 mg/24 hrs. Percentage increase in mean value of microalbuminuria at 24 weeks from baseline (13.16%) was significant. In patients with microalbuminuria and receiving NAC (Group A1), the mean baseline value of microalbuminuria was 209.86±64.32 mg/24 hrs, and at 24 weeks, it was 229.29±60.52 mg/24 hrs. Percentage increase in value at 24 weeks was 9.26% and that was significant. In Group B1, in which the patients were receiving a combination of NAC and taurine, mean baseline microalbuminuria was 225.67±49.60 mg/24 hrs, and at 24 weeks, it was 244.33±53.61 mg/24 hrs. Percentage increase in mean value of the albuminuria as compared to baseline value was 8.27% and this increase in mean value was significant (Table 3).

At 24 week, comparison of decrease in magnitude of microalbuminuria in all three groups (P1, A1, and B1) was insignificant statistically.

In patients with overt proteinuria receiving placebo (Group P2), mean baseline value was 609.67±147.03 mg/24 hrs and at 24 weeks was 657.33±154.20. The percentage increase in overt proteinuria at 24 weeks was 7.82%. This increase in proteinuria was significant at 24 weeks. In patients with overt proteinuria receiving NAC (Group A2), mean baseline value was 628.67±200.83 mg/24 hrs and 24 weeks was 671.83±222.55. The percentage increase in overt proteinuria at 24 weeks was 6.87%. In patients with overt proteinuria receiving a combination of NAC and taurine (Group B2), mean baseline value was 642.75±205.53 and at 24 weeks was 685±218.57 mg/24 hrs.

The percentage increase at 24 weeks was 6.57%. This increase in proteinuria was significant at 24 weeks.

On comparing, decrease in overt albuminuria at 24 weeks in each group (P2, A2, and B2) was statistically insignificant (Table 4).

DISCUSSION

It is well established that ACE inhibitors and ARBs halt the progression of proteinuria in diabetic patients. Studies have shown that oxidative stress leads to progression of glomerulonephritis by producing nicotinamide adenine dinucleotide phosphate oxidase [20,21]. NAC has considerable effects on free radicals and oxidative stress. Plasminogen inhibitors have a role in ECM fixing. Hyperglycemia promotes plasminogen inhibitors and induces free radicals. This phenomenon damages glomeruli in diabetic patients. Lee *et al.* in their study have shown that NAC reduces plasminogen inhibitor levels and decreases free oxygen radicals [22]. Trend toward using NAC due to its antioxidant role has been increasing [23]. Clinically, only one study has been done to demonstrate the therapeutic effects of NAC on proteinuria in diabetic patients [24]. In this study, there was no significant difference of proteinuria before and after NAC administration.

In a study on diabetic rats, taurine, an endogenous antioxidant, has suppressed further increase in urinary protein excretion. In this study, it was also seen that taurine reduced mesangial extracellular matrix expansion and TGF β expression in renal glomeruli. TGF β has been postulated to play an important role in mesangial expansion by accelerating ECM production in diabetic renal tissues. Studer *et al.* [25] had shown that taurine inhibited high glucose-related overexpression of TGF β in cultured mesangial cells in similar fashion to that of NAC. Evidence of beneficial effects of taurine in DN has been accumulating. Ha *et al.* [19] reported that taurine suppresses the expression of TGF β at both mRNA and protein levels. Furthermore, Trachtman *et al.* [21] showed that taurine decreases immunohistochemical staining of Type IV collagen in glomeruli of diabetic rats. However, in our study, there was no significant difference of proteinuria before and after taurine administration.

REFERENCES

- Hasslacher C, Ritz E, Wahl P, Michael C. Similar risks of nephropathy in patients with type I or type II diabetes mellitus. *Nephrol Dial Transplant* 1989;4:859-63.
- Berger M, Mönks D, Wanner C, Lindner TH. Diabetic nephropathy: An inherited disease or just a diabetic complication? *Kidney Blood Press Res* 2003;26:143-54.
- Parving HH. Diabetic nephropathy: Prevention and treatment. *Kidney Int* 2001;60:2041-55.
- Wolf G. New insights into the pathophysiology of diabetic nephropathy: From haemodynamics to molecular pathology. *Eur J Clin Invest* 2004;34:785-96.
- Mason RM, Wahab NA. Extracellular matrix metabolism in diabetic nephropathy. *J Am Soc Nephrol* 2003;14:1358-73.
- Shumway JT, Gambert SR. Diabetic nephropathy-pathophysiology and management. *Int Urol Nephrol* 2002;34:257-64.
- Wolf G, Ziyadeh FN. Cellular and molecular mechanisms of proteinuria in diabetic nephropathy. *Nephron Physiol* 2007;106:26-31.
- Balakumar P, Chakkarwar VA, Krishan P, Singh M. Vascular endothelial dysfunction: A tug of war in diabetic nephropathy? *Biomed Pharmacother* 2009;63:171-9.
- Brown NJ, Vaughan DE. Angiotensin-converting enzyme inhibitors. *Circulation* 1998;97:1411-20.
- Stevens MA, McCullough PA, Tobin KJ, Speck JP, Westveer DC, Guido-Allen DA, *et al.* A prospective randomized trial of prevention measures in patients at high risk for contrast nephropathy: Results of the P.R.I.N.C.E. study. Prevention of radiocontrast induced nephropathy clinical evaluation. *J Am Coll Cardiol* 1999;33:403-11.
- Gare M, Haviv YS, Ben-Yehuda A, Rubinger D, Bdolah-Abram T, Fuchs S, *et al.* The renal effect of low-dose dopamine in high-risk patients undergoing coronary angiography. *J Am Coll Cardiol* 1999;34:1682-8.
- Hall KA, Wong RW, Hunter GC, Camazine BM, Rappaport WA, Smyth SH, *et al.* Contrast-induced nephrotoxicity: The effects of vasodilator therapy. *J Surg Res* 1992;53:317-20.
- Solomon R, Werner C, Mann D, D'Elia J, Silva P. Effects of saline, mannitol, and furosemide to prevent acute decreases in renal function induced by radiocontrast agents. *N Engl J Med* 1994;331:1416-20.
- Seyss C, Foote EF. Calcium-channel blockers for prophylaxis of radiocontrast-associated nephrotoxicity. *Ann Pharmacother* 1995;29:187-8.
- Abizaid AS, Clark CE, Mintz GS, Dosa S, Popma JJ, Pichard AD, *et al.* Effects of dopamine and aminophylline on contrast-induced acute renal failure after coronary angioplasty in patients with pre-existing renal insufficiency. *Am J Cardiol* 1999;83:260-3.
- Conessa EL, Valero F, Nadal JC, Fenoy FJ, López B, Arregui B, *et al.* N-acetyl-L-cysteine improves renal medullary hypoperfusion in acute renal failure. *Am J Physiol Regul Integr Comp Physiol* 2001;281:R730-7.
- DiMari J, Megyesi J, Udvarhelyi N, Price P, Davis R, Safirstein R. N-acetyl cysteine ameliorates ischemic renal failure. *Am J Physiol* 1997;272:F292-8.
- Hamada Y, Miyata S, Nii-Kono T, Kitazawa R, Kitazawa S, Higo S, *et al.* Overexpression of thioredoxin1 in transgenic mice suppresses development of diabetic nephropathy. *Nephrol Dial Transplant* 2007;22:1547-57.
- Hu H, Yu MR, Kim KH. Melatonin and taurine reduce early glomerulopathy in diabetic rats. *Free Radic Biol Med* 1999;26(7-8):944-50.
- Trachtman H, Del-Pizzo R, Futterweit S, Levine D, Rao PS, Valderrama E, *et al.* Taurine attenuates renal disease in chronic puromycin aminonucleoside nephropathy. *Am J Physiol* 1992;262:F117-23.
- Trachtman H, Futterweit S, Maesaka J, Ma C, Valderrama E, Fuchs A, *et al.* Taurine ameliorates chronic streptozocin-induced diabetic nephropathy in rats. *Am J Physiol* 1995;269:F429-38.
- Lee EA, Seo JY, Jiang Z, Yu MR, Kwon MK, Ha H, *et al.* Reactive oxygen species mediate high glucose-induced plasminogen activator inhibitor-1 up-regulation in mesangial cells and in diabetic kidney. *Kidney Int* 2005;67:1762-71.
- Ng TM, Shurmur SW, Silver M, Nissen LR, O'Leary EL, Rigmalden RS, *et al.* Comparison of N-acetylcysteine and fenoldopam for preventing contrast-induced nephropathy (CAFCIN). *Int J Cardiol* 2006 24;109:322-8.
- Saklayen MG, Yap J, Vallyathan V. Effect of month-long treatment with oral N-acetylcysteine on the oxidative stress and proteinuria in patients with diabetic nephropathy: A pilot study. *J Investig Med* 2010;58:28-31.
- Studer RK, Craven PA, DeRubertis FR. Antioxidant inhibition of protein kinase C-signaled increases in transforming growth factor-beta in mesangial cells. *Metabolism* 1997;46:918-25.