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Effects of N-acetyl cysteine on serum lipoprotein (a) and proteinuria in type 2 diabetic patients

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ABSTRACT

Background: About 30-40% of diabetic patients who developed nephropathy have lipoprotein disorders, especially lipoprotein a [Lp(a)], which is related to atherosclerosis.
Objectives: The aim of this study was to investigate the effect of N-acetyl cysteine (NAC) on the serum levels of Lp(a) and amount of proteinuria in a group of type 2 diabetic patients with diabetic nephropathy.
Patients and Methods: A total of 40, type 2 diabetic (T2D) patients, patients with proteinuria, were randomly divided into two groups. The experimental group was treated by NAC (1200 mg/day) for two months in conjunction with conventional treatment for diabetes and hypertension. Control group received routine medications.
Results: No significant change was identified in serum Lp(a) during treatment with NAC (P >0.05). However, NAC decreased the amount of proteinuria, serum triglyceride (TG) level and systolic blood pressure in experimental group compared to the control group (P <0.05).
Conclusions: These findings suggest that treatment with NAC has no significant effect on the serum level of Lp (a). However, it has beneficial effects on the reduction of proteinuria, serum TG level and systolic blood pressure in T2D patients with nephropathy. Further prospective studies are needed to determine its full role.

Implication for health policy/practice/research/medical education:
This study was conducted to investigate the effect of N-acetyl cysteine (NAC) on the serum level of lipoprotein (a) [Lp (a)] and the amount of proteinuria in a group of type 2 diabetic patients .In this investigation, there were no significant changes in the serum Lp (a) during treatment with NAC. However, NAC decreased the amount of proteinuria, serum triglyceride level and systolic blood pressure in type 2 diabetic patients. These findings suggest that NAC may be useful in the treatment of proteinuria in these patients, although further prospective studies are needed to determine its full role.

1. Background

Diabetic nephropathy (DN), is the leading cause of kidney diseases in patients starting renal replacement therapy and affects approximately 40% of type 1 and type 2 diabetic(T2D) patients (1,2). It increases mortality mainly at the result of cardiovascular complications, and is defined by increased urinary albumin excretion in the absence of other renal diseases (1). In spite of widespread investigation on the etiology and treatment of DN, much less attention has been paid to its association with lipopro-

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tein disorders, especially lipoprotein (a) [Lp(a)] which is a marker for atherosclerotic vascular disease progression (2-5). Elevated serum Lp(a) can be accompanied by renal dysfunction, higher risk of atherosclerosis, and dyslipidemia. Moreover, an elevated Lp(a) serum level was associated with albuminuria in diabetic and non-diabetic patients (2). Therefore, reduction of serum level of Lp(a) can decrease the risk of atherosclerotic vascular disease and may improve nephropathy in diabetic patients(6).

Lipoproteins increase in individuals with a higher level of Apo lipoproteins (7). It has been demonstrated that high levels of Lp(a) are associated with brain infarction, coronary artery disease , and nephropathy (2-6). Lp(a) has an important role in the progression of nephropathy in patients with diabetes mellitus (DM) (6-8). Furthermore, serum Lp(a) concentrations in patients with moderate diabetic nephropathy shows a considerable increase in the early stages of renal disease (6).Also the association of Lp(a) with hypertension in diabetic patients has also been shown (9). N-acetyl-cysteine (NAC;C5H9–NO3S) is generally being used as an antioxidant and insulin regulatory agent (10). Various studies have been conducted on the beneficial effects of antioxidant drugs, such as NAC, in reducing the risk of atherosclerosis and its complications. In hemodialysis patients, treatment with NAC caused a reduction in serum Malondialdehyde (MDA) and cardiovascular events (11,12). Most studies have shown that different drugs act on atherosclerosis by several mechanisms such as decreased apoptosis, vasoconstriction, and endothelial dysfunction. NAC is an antioxidant agent and can break the disulfide bonds so reduces the level of Lp(a)(13,14) ,and with this in mind, this drug was used as a treatment for high levels of Lp(a) (13,14).However, the results were different

and less data was published regarding the effect of Lp(a) on progression of nephropathy in T2D, and also modalities for high serum Lp(a).

2. Objectives

The aim of this study was to investigate the effects of N-acetyl cysteine (NAC) on serum levels of Lp(a) in patients with T2D and proteinuria.

3. Patients and Methods

3.1. Patients

Forty patients with T2D participated in this clinical trial. All patients gave informed consent, and all experiments and procedures were performed in accordance with ethics committee of Shahrekord university of medical sciences, Iran. Participants were divided into two aliquots groups I (interventional) and II (non-interventional).

The inclusion criteria were the absence of liver and heart disease, patients who are not on lipid lowering medications, and absence of unwanted or intolerable consequences of NAC during drug use. Study subjects were randomly divided into two groups according to their age, body mass index (BMI), and duration of DM. The first group received NAC (600 mg every 12 hours orally) for 2 months. Patients in the second group received their routine medications.

3.2. Laboratory tests

Blood samples were collected for analysis after overnight fasting at the beginning and after 2 months of the study. Each specimen was collected at room temperature and was allowed to clot over 15 minutes, centrifuged at 2000 g for 15 minutes at 4°C, and separated sera were placed at -70°C until analysis . Total cholesterol (T-chol), plasma TG, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), fasting blood sugar (FBS), Alkaline

Phosphatase (ALP), BUN, creatinine (Cr), and liver enzymes(AST, ALT) were also measured by enzymatic method (Pars Azmun Kit, Iran) using auto-analyzer (BT 3000, France). For all patients, urine analysis was also performed . HbA1c was determined by Biosystem kit (Spain) in accordance with the instructions of the manufacturer. An enzyme immunoassay (EIA) kit was used to measure Lp(a). In addition, blood pressure and blood proteins were measured for 24 hr.

3.3. Statistical analysis

Data were expressed with mean ± SD. Statistical analysis of the data was performed using the Statistics Package for Social Science (SPSS version 13.0, SPSS Inc., Chicago, IL, USA). An independent t-test was used to compare the mean of the variables before and after interventions. Differences were considered significant at P < 0.05 level.

4. Results

Table 1 shows that at the beginning of the study, age, duration of diabetes, and BMI were not significantly different between two groups (p >0.05). Moreover, statistical analysis at the beginning of the study showed that both groups were biochemically and clinically similar (see Table 2) and no significant differences regarding biochemical parameters were seen among groups I and II (p>0.05).

Table 1. Comparison of mean age, duration of diabetes, and BMI in the study groups (groups I and II)

	groups	Mean ± SD	P value
BMI (kg/m²)	I*	27.56 ± 4.12	0.69
	II**	28.09 ± 4.38	
Age (year)	I	62.31 ± 9.32	0.30
	II	59.15 ± 9.49	
Duration of DM (year)	I	9.34 ±5.87	0.84
	II	9 .00 ± 4.99	

*I: Interventional, **II: Control group

Table 3 demonstrates no significant change in serum Lp(a), Cr, BUN, FBS, HbA1c, ALP, ALT,AST, LDL-C, HDL-C and GFR between group I and II after treating with NAC (p > 0.05). Significant reduction of proteinuria, systolic blood pressure, and TG level in group I compared to group II after the treatment with NAC was observed (p < 0.001).

5. Discussion

Previous studies have demonstrated Lp(a) as an independent risk factor for coronary artery disease which increases considerably in patients with diabetic nephropathy (2-9). Furthermore, the level of Lp (a) in patients with macroalbuminuria is higher than patients with microalbuminuria and people with normal-albuminuria (15). It has been reported that Lp (a) increases in patients with diabetic nephropathy , however in a study by Kumari et al. , the results were different(16) . Correlation between serum Lp(a) and hypertension in T2D was reported by Nasri et al. ,while hypertension is an aggravating factor in DN(9). Moreover, a correlation between diabetic nephropathy and increased levels of Lp(a) in diabetic patients was also observed (2,8,15-20). Previous studies proposed that NAC can reduce high levels of Lp (a) due to its antioxidant property (13,21-24). However, Wiklundo et al. found that NAC has no significant effect on the reduction of serum Lp(a) (25). It seems that NAC has limited potency to reduce Lp(a) (25). In addition, the drug dosage and duration of NAC treatment are also important factors which affect the efficacy of this drug (6,25). To investigate the effect of NAC on proteinuria and markers of tubular injury in non-diabetic patients with chronic kidney disease, a placebo-controlled, randomized, open, cross-over study, was conducted on 20 non-diabetic patients with proteinuria with nor-

Table 2. Comparison of mean of variables in both groups before the intervention

Parameters	Groups	Mean ± SD	P value	Parameters	Groups	Mean ± SD	P value
Lp(a) (mg/dL)	I	43.60 ± 12.40	0.91	SGPT (mg/dL)	I	19.50 ± 7.00	0.86
	II	42.70 ± 33.50			II	19.90 ± 6.50	
Systolic BP (mmHg)	I	131.10 ± 19.10	0.91	SGOT (mg/dL)	I	17.50 ± 3.00	0.50
	II	137.70 ± 11.00			II	18.30 ± 4.80	
Diastolic BP (mmHg)	I	87.30 ± 7.50	0.27	ALP (IU/mL)	I	212.70 ± 31.10	0.61
	II	90.00 ± 7.40			II	206.40 ± 44.9	
HbA1c %	I	8.60 ± 2.60	0.30	HDL (mg/dL)	I	43.20 ± 8.80	0.14
	II	7.80 ± 2.00			II	39.20 ± 8.30	
FBS (mg/dL)	I	186.70 ± 86.80	0.93	Cretinine (mg/dL)	I	1.00 ± 0.50	0.74
	II	188.70 ± 46.40			II	1.00 ± 0.13	
TC (mg/dL)	I	214.10 ± 46.50	0.55	Bun (mg/dL)	I	38.00 ± 29.70	0.52
	II	224.30 ± 59.00			II	33.50 ± 9.20	
TG (mg/dL)	I	175.70 ± 89.60	0.09	GFR (ml/min/1.73 m²)	I	86.30 ± 35.5	0.57
	II	232.00 ± 114.60			II	81.20 ±18.00	
LDL (mg/dL)	I	117.80 ± 25.80	0.99	Proteinuria (mg/day)	I	482.20 ±224.2	0.2
	II	117.90 ± 33.80			II	586.90 ± 188.7	

Table 3. Comparison of the mean of variables in the two groups after intervention

Parameters	Groups	Mean ± SD	P value	Parameters	Groups	Mean ± SD	P value
Lp(a) (mg/dL)	I	33.60 ± 11.40	0.29	SGPT (mg/dL)	I	20.00 ± 7.30	0.79
	II	47.20 ± 53.90			II	19.50 ± 4.50	
Systolic BP (mmHg)	I	129.20 ± 5.80	0.04	SGOT (mg/dL)	I	17.70 ± 3.50	0.66
	II	134.00 ± 8.50			II	18.20 ± 3.10	
Diastolic BP (mmHg)	I	84.70 ± 4.50	0.09	ALP (IU/mL)	I	201.20 ± 34.00	0.53
	II	88.00 ± 7.10			II	193.80 ± 38.60	
HbA1c %	I	7.90 ± 1.90	0.89	HDL (mg/dL)	I	41.92 ± 7.40	0.12
	II	7.80 ± 1.40			II	38.40 ± 6.50	
FBS (mg/dL)	I	155.10 ± 68.00	0.51	Cretinine (mg/dL)	I	1.00 ± 0.30	0.87
	II	167.10 ± 45.10			II	1.00 ± 0.15	
T-Chol (mg/dL)	I	215.50 ± 48.10	0.79	Bun (mg/dL)	I	36.80 ± 26.50	0.44
	II	211.20 ± 56.00			II	32.00 ± 8.90	
TG (mg/dL)	I	169.70 ± 76.30	0.04	GFR (ml/min)	I	83.90 ± 29.40	0.71
	II	228.80 ± 99.80			II	80.80 ± 22.50	
LDL (mg/dL)	I	119.40 ± 25.90	0.88	Proteinuria (mg/day)	I	381.70 ± 191.70	0.04
	II	118.00 ± 32.10			II	499.00 ± 170.30	

mal or decreased kidney function. It was found that NAC has no effect on proteinuria, surrogate markers of tubular injury or renal fibrosis in non-diabetic patients with chronic kidney disease (26). The possible effect of NAC on the reduction of proteinuria may be due to its antioxidant prop-erty (25-28). Also, the effect of NAC on drug-induced HTN as a result of decreased blood pressure was investigated (28). In this regard, the positive effect of NAC on the reduction of HTN , was shown (23). Likewise, reduced blood pressure in the NAC treated group was observed

in our study as well. The combinational effects of L-argentine and NAC on the level of blood pressure in patients with diabetic nephropathy was studied by Martina et al. (29). They showed that L-arginine, is precursor for nitric oxide bio-synthesis. The combination of L-argentine and NAC resulted in reduction of both systolic and diastolic blood pressure. Furthermore, a significant decrease in other risk factors of coronary artery disease such as total and LDL-C levels was observed (29). Also, our study showed the reduction of SBP and serum TG levels compared to the control group. Similar result was observed on animal studies which showed the serum TG -lowering effect of NAC (30). In a clinical study on NAC, similar reduction of TG and T-Chol, along with HDL-c elevation, was observed (31),however, no significant change in HDL-C was found in our study.

6. Conclusions

The results of this study suggest that treatment with NAC does not decrease the serum level of Lp (a) could reduce the amount of proteinuria, serum TG level, and SBP in patients with T2D and proteinuria. Further studies are needed to clarify the significance of our findings.

Authors' contributions

HR designed and performed the research. FG analyzed data and wrote some parts of the manuscript. HR prepared the manuscript.

Conflict of interest

The author declared no competing interests.

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