INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-7, Issue-1, Jan-Mar-2016 Coden IJABFP-CAS-USA Copyrights@2016

Received: 10th Dec-2015 *Revised: 28th Dec -2015* *Accepted: 4th Jan-2016*

Research article

THE EFFECT OF STATIN GROUP ON ALBUMIN CREATININE RATIO IN TYPE 2 DIABETIC PATIENT

Metwally N.A[1*] and Khalil A.KH[1]

[1]Department of Basic medical science, College of Medicine, Dar Al Uloom University, Riyadh, Kingdom of Saudi Arabia.

ABSTRACT

Introduction: The earliest clinical evidence of nephropathy is the appearance of low but abnormal levels of albumin in the urine, referred to as microalbuminuria, which is defined as a level of urinary albumin excretion in excess of the upper limit of reference range for non-diabetic subjects. Once overt nephropathy sets in, progression of nephropathy can only be slowed down but cannot be reversed with the present treatment.

Material and methods: 20 type 2 diabetic patients with increase Albumin excretion ratio (AER), 11 type 2 diabetic patients with normal AER and 10 healthy control subjects were enrolled in the study after written consent. Full medical history and clinical examination was done .Investigations of the blood for random blood glucose and two hours post prandial was done. Random urine samples were collected from all participants for determination of albumin to creatinine ratio (ACR). Urine microalbuminuria was determined by Immunoturbidometry-Latex method using BioSystems kit.

Results: ACR level showed significant reduction in the two studied patients groups after fluvastatin therapy (p =0.005; for group I) and (P=0.044; for group II) compared to corresponding values before treatment.

Conclusion: Statin is one of the drugs which has positive effect on the nephropathy of diabetes type 2.

Recommendation**:** The use of high doses of fluvastatins in DM patients, be considered to be safe and well tolerated, however further investigations are needed to evaluate the effect of high dose statins for a large period of time.

Key Words: AER (Albumin excretion rate), ACR (albumin creatinine-ratio), Diabetes, Nephropathy, Microalbuminuria, Statin

*Corresponding author: Noha Adel Metwally, [1]Department of Basic medical science, College of Medicine, Dar Al Uloom University, Riyadh, Kingdom of Saudi Arabia, E-mail: nohamtwally@gmail.com

Tel: 00966564006777

INTRODUCTION

The earliest clinical evidence of nephropathy is the appearance of low but abnormal levels of albumin in the urine, referred to as microalbuminuria, (Mark E et al, 2004) which is defined as a level of urinary albumin excretion in excess of the upper limit of reference range for non-diabetic subjects and which is classically undetectable by Albustix Dipstix test. Albustix shows a positive result at around 250-350 mg/L. (Shankar P 2004) Without specific interventions, 20–40% of type 2 diabetic patients with microalbuminuria progress to overt nephropathy, but by 20 years after onset of overt nephropathy, only about 20% will progress to end stage renal disease (ESRD).

(Mark E et al, 2004) In addition to its being the earliest manifestation of nephropathy, albuminuria is a marker of greatly increased cardiovascular morbidity and mortality for patients with either type 1 or type 2diabetes.

Thus, the finding of microalbuminuria is an indication for screening for possible vascular disease and aggressive intervention to reduce all cardiovascular risk factors (e.g., lowering of LDL cholesterol, antihypertensive therapy, cessation of smoking, institution of exercise, etc.). (Mark E et al, 2004, Chang C et al, 2011) Once overt nephropathy sets in, progression of nephropathy can only be slowed down but cannot be reversed with the present treatment. (Shankar P 2004) Early renal changes in diabetes are characterized by kidney enlargement, glomerular hyperfiltration and increased synthesis of extracellular matrix. (Khamaisi M et al, 2003) One of the dominant histological features of the abnormal kidney from diabetics is the expansion of the mesangium, and particularly over secretion and deposition of extracellular matrix (ECM) proteins (such as collagen IV, fibronectin and laminin B1). In addition, an imbalance in the control of mesangial cells (MC) proliferation appears to play an early and crucial role in the initiation and progression of glomerulosclerosis. (Geoffroy K et al, 2005) Diabetic retinopathy (DR) is the leading cause of blindness in the Western world and is characterized by abnormal angiogenesis which results in new vessels that are often immature and play a pathological role in retinopathy, contributing to both vitreous hemorrhage and fibrosis. In addition, increased vascular permeability leading to plasma leakage accounts for the development of macula edema, disrupting visual function. (Nakagawa T et al, 2009) Diabetic nephropathy is also characterized by abnormal angiogenesis driven by several factors, including tissue ischemia and hyperglycemia. This abnormal angiogenesis results in new vessels that are often immature and play a pathological role in nephropathy. (Nakagawa T et al, 2009, Advani A et al, 2012) Intriguingly, the progression of both diabetic retinopathy and nephropathy is altered by vascular growth factor signaling through receptor tyrosine kinases, specifically involving the vascular endothelial growth factor (VEGF-A) and angiopoietin families.(Nakagawa T et al, 2009) 6)

The study was designed to evaluate the alteration of ACR of diabetic patients after using statin drug.

SUBJECTS AND METHODS

Subjects

After the approval of the ethics committee of the medical research institute .The present study was conducted on 41 subjects categorized as follows:

Group (I): Included 20 type 2 diabetic patients with increased urinary albumin excretion rate (AER).

Group (II): Comprised 11 type 2 diabetic patients with normal urinary albumin excretion rate.

Control group: Ten healthy volunteers of comparable age and sex to the patients group were served as controls.

All patients were recruited from the internal medicine department in the Medical Research Institute, Alexandria University. Subjects with any conditions other than diabetic complications that can affect vascular endothelial growth factor (VEGF-A) and or angiopoietin -2 were excluded such as tumors, collagenic disorders and hepatitis C. Written consents were obtained from all participants before entry in the study.

METHODS

The followings were done to all the enrolled subjects:

Detailed history taking and full clinical examination with special stress on duration and treatment of diabetes, any complaints due to diabetic complications and determination of blood pressure.

Blood sampling

1. Fasting blood sample

Ten milliliters venous blood sample was taken from each subject after 12-14 hours fasting. Part of the sample was withdrawn on EDTA-disodium salt for determination of (HbA1c) while the rest was left for clotting. Serum was rapidly and carefully removed from the red cells by centrifugation at 1500 rpm for ten minutes. It was then divided as follows:

- An aliquot was immediately used for determination of some laboratory investigations such as: Fasting blood glucose, creatinine, uric acid concentrations, Aspartate transaminase (AST), Alanine taransaminase (ALT), Creatine kinase (CK), activities and lipid profile.
- Another aliquot was divided into two parts in an eppendorf tubes one of them stored at -20˚C for serum VEGF-A determination. And the other stored at -80˚C for ANGPT-2 determination. Both thawed just before the assay.

2. Post prandial blood sample

Two milliliters venous bloods were taken from all studied subjects exactly 2 hours after breakfast. Serum was separated and used for post prandial serum glucose estimation.

Urine sampling

Random urine samples were collected from all participants for determination of albumin to creatinine ratio (ACR).

Determination of urine microalbuminuria: (Edmund L et al, 2006)

Urine microalbuminuria was determined by Immunoturbidometry-Latex method using Bio Systems kit. Albumin in the urine sample causes agglutination of the latex particles coated with anti-human albumin. The increase of the particles agglutination is proportional to the albumin concentration and can be measured immunoturbidometrically.

Albumin/creatinine ratio:

$$\text{ACR (mg/g)} = \frac{\text{Microalbumin in urine (mg/L)} \times 1000}{\text{creatinine in urine (mg/dl)} \times 10}$$

The urine creatinine value is multiplied by 10 to convert mg/dL to mg/L, then divide the urine albumin value by the urine creatinine value to arrive at the ratio, then multiply by 1000 to express the value as (mg albumin/g creatinine).

RESULTS

Table 1 showed demographic data of the study groups: The present study included 11 diabetic patients with normal albumin excretion rate (AER),their age ranged between 23-42 years with a mean of 30.55 ± 1.91 years (group I). This group included 5 (45.5%) males and 6 (54.5%) females. Group II included 20 diabetic patients with increased AER, their age ranged between 44-66 years with a mean of 54.75 ± 1.53 years .This group included 6 (30.0%) females and 14 (70.0%) males. Ten healthy subjects are included as controls, their age ranged between 20-67 years with a mean of 38.10 ± 5.16 years. This group included 5 (50%) males and 5 (50%) females as shown in table (1).

The duration of diabetes mellitus in diabetic patients with normal and increased albumin excretion rate is shown in table (2) .In group (I) the duration of diabetes mellitus ranged between 2.0 – 9.0 years with a mean of 5.09 ± 0.69 and in group (II) the duration of diabetes mellitus ranged between 8.0 – 35.0 years with a mean of 20.30 ± 1.62.

Table-1: Age and sex of diabetic patients with normal and increased albumin excretion rate (AER) and of control subjects.

	Diabetic patients with normal AER (n = 11)		Diabetic patients with increased AER (n = 20)		Controls (n = 10)	
	No.	%	No.	%	No.	%
Sex						
Male	5	45.5	14	70.0	5	50.0
Female	6	54.5	6	30.0	5	50.0
Age						
Range	23.0 – 42.0		44.0 – 66.0		20.0 – 67.0	
Mean ± SE	30.55 ± 1.91		54.75 ± 1.53		38.10 ± 5.16	
Median	30.0		54.50		34.0	

Table-2: Duration of diabetes mellitus in diabetic patients with normal and increased albumin excretion rate (AER).

	Diabetic patients with normal AER (n = 11)	Diabetic patients with increased AER (n = 20)
Duration of DM		
Range	2.0 – 9.0	8.0 – 35.0
Mean ± SE	5.09 ± 0.69	20.30 ± 1.62
Median	5.0	20.0

Individual data and mean values ± SE of urinary albumin / creatinine ratio (ACR) in diabetic patients (group I and II) before and after fluvastatin therapy and of control group are shown in table (3). The statistical analysis of the data is shown in tables (4, 5).

The mean value of ACR in patients with normal AER was12.78 ± 1.26 mg/g before starting therapy and 9.55± 1.08 mg/g after therapy, while in patients with increased AER the mean value was 79.78±21.98 mg/g before therapy and 59.97 ± 13.35 mg/g after treatment. In control subjects, the mean value of ACR was 14.30 ±1.04 mg/g.

Table-3: Urinary albumin/creatinine ratio [(ACR), mg/g] of diabetic patient with normal and increased albumin excretion rate (AER) before and after fluvastatin therapy and of control subjects.

No.	Diabetic patients with normal AER (n = 11)		Diabetic patients with increased AER (n = 20)		Controls (n = 10)
	Before treatment	After treatment	Before treatment	After treatment	
1	7.9	7.9	34	27.8	12
2	7.1	6.4	66	63.2	14
3	14.4	5.3	61.2	48.7	17
4	12	11.2	37.2	20.2	20
5	7.9	6.8	53.6	22.6	12
6	9.2	7.8	487	90	16
7	17.8	14.6	70	302	18
8	13.7	8.5	107	38	13
9	15.7	7.4	30	29.7	11
10	18.2	15.3	61.7	62.4	10
11	16.7	13.9	60	69.5	
12			90.7	66	
13			60	40.5	
14			59.5	43.7	
15			25	50.4	
16			40.2	57.9	
17			70	50.8	
18			80	44.8	
19			24.7	23.6	
20			77.7	47.5	
Mean	12.78	9.55	79.78	59.97	14.30
SE	1.26	1.08	21.98	13.35	1.04

ACR levels showed insignificant difference between the diabetic group of patients with normal AER before starting fluvastatin therapy as compared to the corresponding mean value of control group (P=0.438), while it showed significant elevation in diabetic group of patients with increased AER before starting fluvastatin therapy as compared to the corresponding mean value of control group, (P<0.001) as shown in (table 4). Significant elevation in ACR level was observed in diabetic patients of group (II) before starting fluvastatin therapy as compared to the corresponding mean value of diabetic patients of group (I), (P<0.001) as shown in table (4). ACR level showed significant reduction in the two studied patients groups after fluvastatin therapy (p=0.005; for group I) and (P=0.044; for group II) compared to corresponding values before treatment, table (4). ACR level was significantly high in all studied diabetic patients group before starting fluvastatin therapy as compared to corresponding values of control group, as shown in table (5). ACR level showed significant reduction in all studied diabetic patients group after fluvastatin therapy compared to the corresponding values before treatment (p<0.034) as shown in table (5).

Table-4: Statistical analysis of urinary albumin /creatinine ratio (ACR) [@] in the three studied groups.

	Diabetic patients with normal albumin excretion rate (n = 11)		Diabetic patients with increased albumin excretion rate (n = 20)		Controls (n = 10)
	Before treatment	After treatment	Before treatment	After treatment	
ACR					
Range	7.10 – 18.20	5.30 – 15.30	24.70 – 487.0	20.20 – 302.0	10.0 – 20.0
Mean ± SE	12.78 ± 1.26	9.55 ±1.08	79.78 ±21.98	59.97 ± 13.35	14.30 ±1.04
Median	13.70	7.90	60.60	48.10	13.50
p	0.005*		0.044*		
p1	0.438	0.011*	<0.001*	<0.001*	
p2			<0.001*	<0.001*	

P: vs. values before treatment within the same group (Paired t-test).
p_1:vs. control (Mann Whitney tests).
p_2: vs. corresponding values of the other patient's group (Mann Whitney test).
*: $p \leq 0.05$ considered statistically significant
@: values are expressed in mg/g.

Table-5: Statistical analysis of urinary albumin /creatinine ratio (ACR) [@] of all studied diabetic patients and of controls.

	All diabetic patients (n = 31)		Controls (n = 10)
	Before treatment	After treatment	
ACR ratio			
Range	7.10 – 487.0	5.30 – 302.0	10.0 – 20.0
Mean ± SD	56.0 ± 84.78	42.08 ± 53.50	14.30 ± 3.30
Median	37.20	29.70	13.50
p_1	<0.034*		
p_2	0.007*	0.052	

p_1: vs.values before treatment within the same group (Wilcoxon signed ranks test)
p_2:vs control (Mann Whitney test)

DISCUSSION

In recent years, the inhibition of 3-hydroxy-3-methylglutaryl CoA reductase by statins has demonstrated beneficial effects in different models of progressive renal failure. It is interesting that some of the beneficial effects of statins can be seen independent of the cholesterol reduction. (Roberto T et al, 2006, Oda H et al, 1999) In the present work urinary ACR was significantly decreased in all diabetic patients after treatment with fluvastatin when compared to corresponding values before treatment. Albuminuria is one of the clinical parameters for diagnosing renal damage, particularly in cases of DN, and it has been reported to be a risk factor and predictor of ESRD. Therefore, the reduction of albuminuria is a major goal in the treatment of DN. (Annunziata L et al, 2012) Similarly in a previous study it was reported that, in dyslipidemic patients with CKD using fluvastatin for 2 months reduced UAE in patients with microalbuminuria was observed .These results suggest that fluvastatin might be potentially effective to improve renal function in addition to its cholesterol lowering effect. (Teruo I et al, 2011). One trial assessed albuminuria in terms of urinary albumin to creatinine ratio and found a significant reduction as a result of statin therapy, (Sabrina S et al, 2006). and other study found that statins reduce albuminuria by 47 and 48% in people with >300□mg/24□h and 30–300□mg/24□h of albumin excretion at baseline, respectively. (Douglas K et al, 2006) In a meta-analysis of randomized, placebo-controlled trials in adults, statins were associated with statistically significant reductions in pathologic levels of albuminuria and proteinuria.

These effects may be due to the ability of statins to improve endothelial dysfunction, supported by other biological evidence of such an effect. (Douglas K et al, 2006) Most studies have shown that the vasoconstriction associated with endothelial dysfunction can be attenuated or abolished with statin therapy. (Egashira K et al, 1994, Treasure CB et al, 1995, Tsunekawa T et al, 2001) This improvement in endothelial function can be seen within 6 weeks (Dupuis J et al, 1999) and partly results from an increase in endothelial nitric oxide activity by activation of nitric oxide release and concurrent inactivation of superoxide. (John S et al, 1998). A reduction in serum oxidized LDL cholesterol level may contribute to improvements in endothelial function since oxidized LDL cholesterol downregulates endothelial nitric oxide synthase activity. (Douglas K et al, 2006) Since lipid lowering by statins reduces lipid trapping in renal tissues, the lipid lowering itself may contribute to renoprotective effects. However, some of the renoprotective effects of statins can be seen independent of the cholesterol reduction (Teruo I et al, 2011). Experimental data suggest that statins reduce proteinuria at least in part by reducing inflammation and fibrosis in the renal interstitium, seemingly through actions on monocyte chemotactic protein-1 and TGF-α. (Sabrina S et al, 2006). In an elegant study by Zoja *et al.* it was shown that a combined angiotensin-converting enzyme inhibitor and statin therapy had a remarkable antiproteinuric effect with a significant improvement in renal function. Drug combination limited glomerulosclerosis, tubular damage, and interstitial inflammation, compared with placebo or drugs alone.

In vitro studies have established clearly that statins influence important intracellular pathways that are involved in the inflammatory and fibrogenic responses, which are common components of many forms of progressive renal injury. [(10)]Statins also inhibit proliferation of cultured mesangial cells and renal epithelial tubular cells through their capability to suppress the formation of intermediate metabolites of the mevalonate pathway, particularly the nonsterol isoprenoids, which seem to be essential in cell replication. (Roberto T et al, 2006, O'Donnell MP et al, 1993).

Finally, the putative benefits of statins on kidney function may be attributable to their effects on proteinuria, which is a powerful predictor of kidney function loss. (Sabrina S et al, 2006).

Recommendation

The use of high doses of fluvastatins in DN patients, conducted to our study could be considered to be safe and well tolerated, however further investigations are needed to evaluate the effect of using high dose statins for a large period of time with a large sample size on the studied parameters to evaluate the safety and tolerability of the drug.

REFERENCES

Mark E, Molitch M, Ralph A, Marion J, Franz MS, (2004). Nephropathy in diabetes. Diabetes Care 27:79-83.

Shankar P. (2004). Preventing Renal Disease in Type 2 Diabetes Mellitus. JIACM 5(2): 160-65.

Chang C, Chang CH, Yang W, Huang J , Yen T. (2011). Resveratrol retards progression of diabetic nephropathy through modulations of oxidative stress, proinflammatory cytokines, and AMP-activated protein kinase. Journal of Biomedical Science 18:47-57.

Khamaisi M, Bieke F. Schrijvers, Vriese An, Raz I and Flyvbjerg A. (2003). The emerging role of VEGF in diabetic kidney disease. Nephrol Dial Transplant 18: 1427–1430.

Geoffroy K, Troncy L, Wiernsperger N, Lagarde M , El Bawab S. (2005). Glomerular proliferation during early stages of diabetic nephropathy is associated with local increase of sphingosine-1-phosphate levels. The Journal of Rapid Publications of Short Reports in Molecular Bioscience 579(5): 1249-54.

Nakagawa T, Kosugi T, Haneda M ,Christopher J, Rivard. (2009). Abnormal Angiogenesis in Diabetic Nephropathy. Diabetes 58:1471-78.

Advani A, Gilbert R. (2012). The endothelium in diabetic nephropathy. Seminars in Nephrophrology 32(2):199-207.

Edmund L, David J, Christopher P.Kidney Function Tests. In: Tietz (2006). Text Book of Clinical Chemistry and Molecular Diagnostics. Burtis CA, Ashwood ER, Bruns DE. (eds). 4th ed. Elsevier Saunders Company, St Louis. pp. 813-5.

Roberto T, Alessandro R, Giuseppe L. (2006). Lipids and Renal Disease. J Am Soc Nephrol 17: 145–7.

Oda H, Keane WF. (1999). Recent advances in statins and the kidney. Kidney Int Suppl 71: 2–5.

Annunziata L, Simona P, Pietro T. **(**2012). Mass Spectrometry for Diabetic Nephropathy Monitoring: New Effective Tools for Physicians. ISRN Endocrinology 3:1-13.

Teruo I, Hideo I , Tsukasa N , Shichiro A, Isao T, (2011). Potential benefit of statin therapy for dyslipidemia with chronic kidney disease: fluvastatin renal evaluation trial (FRET). Intern Med 50: 1273-78.

Sabrina S, Natasha W, Linda F, Marcello T. (2006). Statins for Improving Renal Outcomes: A Meta-Analysis. J Am Soc Nephrol 17: 2006–16.

Douglas K, O'Malley PG, Jackson JL. (2006). Meta-analysis: the effect of statins on albuminuria. Ann Intern Med 145: 117–24.

Egashira K, Hirooka Y, Kai H, Sugimachi M, Suzuki S, Inou T, (1994). Reduction in serum cholesterol with pravastatin improves endothelium dependent coronary vasomotion in patients with hypercholesterolemia. Circulation 89:2519-24.

Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, et al. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. N Engl J Med 1995; 332: 481-7.

Tsunekawa T, Hayashi T, Kano H, Sumi D, Matsui-Hirai H, Thakur NK, (2001). Cerivastatin, a hydroxymethylglutaryl coenzyme A reductase inhibitor, improves endothelial function in elderly diabetic patients within 3 days. Circulation 104:376-9.

Dupuis J, Tardif JC, Cernacek P, The´roux P. (1999). Cholesterol reduction rapidly improves endothelial function after acute coronary syndromes. The RECIFE (reduction of cholesterol in ischemia and function of the endothelium) trial. Circulation 99:3227-33.

John S, Schlaich M, Langenfeld M, Weihprecht H, Schmitz G, Weidinger G. (1998). Increased ioavailability of nitric oxide after lipid-lowering therapy in hypercholesterolemic patients: a randomized, placebo-controlled, double-blind study. Circulation 98:211-6.

Zoja C, Corna D, Rottoli D, Cattaneo D, Zanchi C, Tomasoni S, Abbate M, Remuzzi G. (2002). Effect of combining ACE inhibitor and statin in severe experimental nephropathy. Kidney Int 61: 1635– 45.

O'Donnell MP, Kasiske BL, Kim Y, Atluru D, Keane WF. (1993). Lovastatin inhibits proliferation of rat mesangial cells. J Clin Invest 91: 83–7.

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com