Effects of Folic Acid Supplementation on Proteinuria in Type 2 Diabetic Patients with Overt Proteinuria.

Sarasadat Moghadasimousavi[1], Nader Nouri–Majalan [2*], Roghayyeh Masoumi-Dehshiri[3]

1- Resident of internal medicine, Division of Nephrology, Department of Internal Medicine, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran.
2- Associate Professor, Division of Nephrology, Department of Internal Medicine, Faculty of medicine, Shaheed Sadoughi University of Medical Sciences and Health Services, Yazd, Iran.
3- Health Policy Research Center, Shaheed Sadoughi University of Medical Sciences and Health Services, Yazd, Iran.

***Correspondence:**
Nader Nouri-Majalan, Department of Internal Medicine, Faculty of medicine, Shaheed Sadoughi University of Medical Sciences and Health Services, Yazd, Iran.
Tel: (+98) 9131526380
Fax: (+98-351) 8224100
Email: dr_nori_majelan@yahoo.com

Received: 25 July 2012
Accepted: 27 August 2012

Abstract

Objective: An association between high homocysteine levels and proteinuria in diabetic nephropathy has been shown. On the other hand, supplementation with folic acid lowers homocysteine concentration. We have studied the effects of folic acid supplementation on proteinuria in type 2 diabetic patients with overt proteinuria.

Materials and Methods: Forty five (29 men and 16 women) type 2 diabetic patients with overt proteinuria were randomized into 2 groups. One group was treated with folic acid for 6 months (22 patients), and the other was not (control group, 23 patients). Twenty-four hours proteinuria, creatinine clearance, and serum homocysteine were measured at baseline and 6 months of treatment.

Results: In the treatment group, baseline proteinuria was 2927 ± 2858 mg/day, and after 6 months of folic acid treatment, there was a significant decrease in proteinuria to 2049 ± 1641 mg/day ($P < 0.05$). For the control group, there was no change of proteinuria after 6 months of follow-up ($P = 0.9$). Serum homocysteine levels were significantly decreased in subjects treated with folic acid, from 19.2 ± 9.46 µmol/L to 16 ± 5.6 µmol/L; $P < 0.05$). There were no significant differences in creatinine clearance at the end of the study comparing the two groups.

Conclusion: Supplementation with folic acid decreases proteinuria in type 2 diabetic patients with overt proteinuria and that this action may be mediated through reduced concentrations of serum homocysteine. However, for evaluation of the effect of folic acid on renal function, further studies with longer follow-up are needed.

Keywords: Folic acid, Proteinuria, Diabetic nephropathy, Homocysteine

Introduction

Nephropathy associated with type 2 diabetes is the most frequent cause of end stage renal disease. The increased susceptibility of diabetic patients with nephropathy can be explained by risk factors such as genetic susceptibility (1), increased glomerular filtration rate (2), obesity (3), and glycemic control. Activation of the renin-angiotensin system (RAS) has a major role in the pathogenesis of diabetic nephropathy. Inhibition of this system by angiotensin-

converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) currently is the mainstay of diabetic nephropathy management (4). However, despite RAS inhibition, diabetic nephropathy progresses to end stage renal disease in a large proportion of patients (4, 5). Therefore a novel treatment is needed. An association between high homocysteine (Hcy) levels and proteinuria in diabetic nephropathy has been shown in previous studies (6, 7). On the other hand, supplementation with folic acid lowers Hcy concentration (8). Therefore, folic acid may decrease proteinuria in diabetic nephropathy.

We have studied the effects of folic acid supplementation on proteinuria in type 2 diabetic patients with overt proteinuria.

Materials and Methods

Between January and December 2012, we evaluated forty-five consecutive type 2 diabetes mellitus patients with overt diabetic nephropathy, defined as daily proteinuria greater than 0.3 g. We excluded patients with serum creatinine>4.5 mg/dL, hemodialysis patients and patients already on folic acid or B vitamins supplementation. The study protocol was approved by the ethics committee of our institution, and informed consent was obtained from each patient.

Patients were randomized to receive 5 mg/d folic acid, or no folic acid (control group). Demographic data, blood pressure, creatinine clearance (CrCL), and other biochemical profiles such asserum creatinine, fasting blood sugar, and serumHcy concentrations were evaluated at baseline and after 6 months of treatment. CrCL was calculated with Cockcroft-Gault equation. Serum creatinine and fasting blood sugar concentrations were measured by the standard methods. Serum samples were drawn after 12-hour fasting. Serum Hcy concentrations were measured by enzyme-linked immunosorbent assay (IBL Company, Hamburg, Germany). Twenty-four hour urine collections were obtained with measurement of urine protein and creatinine. Measurement of creatinine excretion permitted assessment of completeness of the 24-hours urine collection. Any adverse event considered to be related to the use of folic acid such as flushing, bronchospasm, and skin rash were recorded during the follow-up assessment.

Statistical Analysis

All statistical analyses were performed using the SPSS Chicago, IL, US software version 13. Unless otherwise stated, values are expressed as mean±SD. Categorical data were compared by means of chi-square test, and continuous variables, by means of Student t-test. Comparison of various parameters between baseline and different intervals was performed by means of paired Student t-test. Statistical significance is defined as 2-tailed P less than 0.05.

Results

Between January and December 2012, a total of 45 patients were enrolled in the study. Baseline characteristics, demographic, laboratory parameters, and medications used in our study patients are listed in Table 1. There were no statistically significant differences between the 2 groups in terms of demographics data, blood pressure, CrCL, serum Hcy concentrations, 24-hours proteinuria, and proportion of patients using ACE inhibitors, ARBs, and nondihydropyridine calcium channel blockers.

Twenty-three patients in control group and 22 patients in treatment group completed the study. In the treatment group, baseline proteinuria was 2927±2858 mg/day, and after 6 months of folic acid treatment, there was a significant decrease in proteinuria to 2049±1641 mg/day (P<0.05; Fig. 1). For the control group, proteinuria at baseline was 1849±1444 mg/day. There was no change after 6 months of follow-up (1828±1981 mg/day; P=0.9). Comparison of proteinuria showed no significant difference between the two groups (P=0.7).

Table 1- Baseline characteristics of subjects in control and folic acid groups

Characteristics	Control group (n=23)	Folic acid group (n=22)	P value
Age (year)	58±8.9	57±8.6	NS
Males proportion [n (%)]	14 (31%)	15(33%)	NS
Body mass index (kg/m^2)	21±3	22±3.1	NS
Blood pressure (mmHg)			
Systolic	150±21	147±15	NS
Diastolic	85±10	82±11	NS
Serum homocysteine (µmol/L)	18.4±5.87	19.2±9.46	NS
Serum creatinine (mg/dL)	1.7±0.59	2.1±0.77	NS
Creatinine clearance (mL/min)	68±40	62±37	NS
24-hours proteinuria (mg)	1849±1441	2927±2858	NS
Fasting blood sugar (mg/dL)	165±53	185±100	NS
Medications [n (%)]			
ACEIs	8 (18%)	11 (24%)	NS
ARBs	22(50%)	20(44%)	NS
Calcium channel blockers	11(25%)	10(23%)	NS

ACEI, angiotensin converting enzyme inhibitors; ARBs, angiotensin II receptor blockers
NS= Not Significant

When we compared CrCl of the two groups after treatment for 6 months, there was no significant difference in CrCl (51±33 mL/min in the treatment group vs 58±32 mL/min in the control group; P=0.4) (Fig. 2). However, significantly, deterioration of CrCl in each group was observed when compared with baseline CrCl (62±37 vs 51±33 mL/min in the treatment group; P <0.05; and 68±40 vs 58±32 mL/min in the control group; P<0.05) (Fig. 2).

Baseline serum Hcy levels were similar in the groups (19.2±9.46 µmol/L in the treatment group and 18.4±5.87 µmol/L in control group; P=0.7). Serum Hcy levels at the end of folic acid treatment was 16 ± 5.6 µmol/L (P<0.05), and for the control group, 20±10.6 µmol/L after 6-month follow-up (P=0.2) (Fig. 3). There was also no statistical difference between the 2 groups at the end of the study (P=0.13).

Serum FBS was similar between the two groups at the end of the study (142±66 mg/dL in the treatment group and 157±62 mg/dL in control group; P=0.4). We did not find any adverse effects such as allergic reaction, bronchospasm, flushing and rash by folic acid treatment.

Discussion

We showed that proteinuria decreased in the treatment group after folic acid supplementation. However, proteinuria was not significantly different from the control group. We also found folic acid is able to decrease Hcy level.

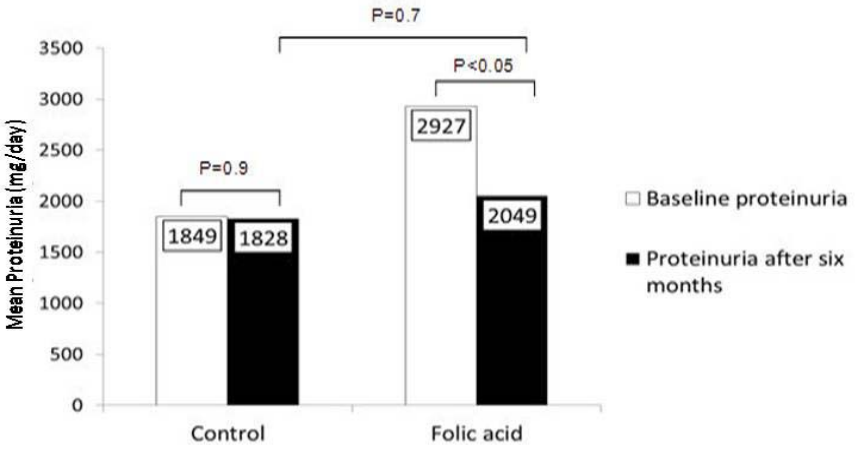

Figure 1: Mean proteinuria (mg/day) at baseline and at the end of treatment

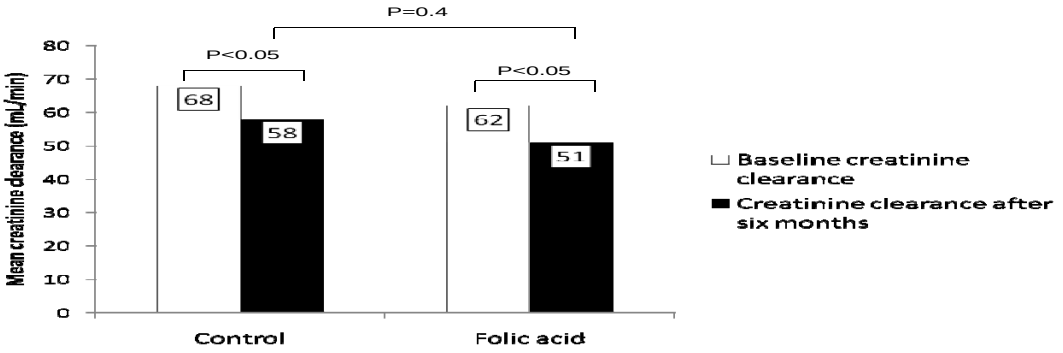

Figure 2: Mean creatinine clearance (mL/min) at baseline and at the end of treatment.

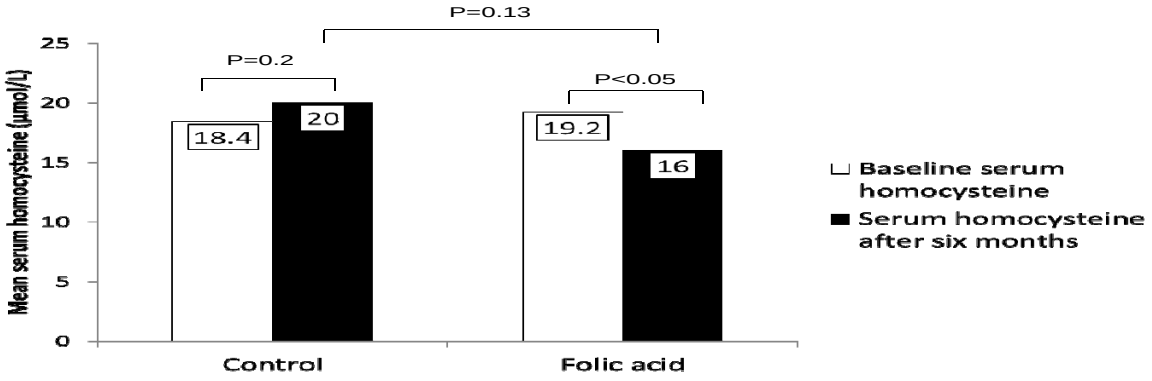

Figure 3: Mean serum homocysteine (µmol/L) at baseline and at the end of treatment.

To our knowledge, this is the first study regarding use of folic acid in order to decrease proteinuria through its ability to lower serum Hcy level in overt diabetic nephropathy. The precise mechanism is not known, but may be related to an association between high Hcy levels and proteinuria in diabetic nephropathy. Previous studies showed in type 2 diabetic patients with overt nephropathy had higher Hcy compared with no proteinuria (7, 9, 10). Hcy may decrease proteinuria through the hypothesized mechanism of endothelial damage (11). It is wellknown that ACE inhibitors, ARBs, and nondihydropyridine calcium channel blocker decrease proteinuria in diabetic nephropathy and they could confound our result. However, there were no differences in the use of these medications in the two groups at the beginning and end of the study.

We showed that folic acid has no effect on renal function. However, in a recent randomized placebo-controlled trial of participants with diabetic nephropathy and stages 1 to 3 of chronic kidney disease, the use of high doses of B vitamins (containing 2.5 mg/d of folic acid, 25 mg/d of vitamin B6, and 1mg/d of vitamin B12) compared with placebo, resulted in a greater decrease in CrCl (12). The discrepancy may be due to the difference between the treatments. We treated patients only with folic acid while they used combination of high doses of B vitamins. Also, they followed patients for 3 years, but our follow-up was shorter (6 months).

Conclusion

In summary, we found that supplementation with folic acid decrease proteinuria in type 2 diabetic patients with overt proteinuria and that this action may be mediated through reduced concentrations of serum Hcy. However, for evaluation of the effect of folic acid on renal function, further studies with longer follow-up are needed.

Acknowledgments

The authors wish to thanks the patients for their assistance for performing this study.

References

1. Adler S. Diabetic nephropathy: Linking histology, cell biology, and genetics. Kidney Int. 2004; 66(5):2095-106.
2. Rudberg S, Persson B, Dahlquist G. Increased glomerular filtration rate as a predictor of diabetic nephropathy--an 8-year prospective study. Kidney Int. 1992; 41(4):822-8.
3. Tapp RJ, Dip G, Shaw JE, Zimmet PZ, Balkau B, Chadban SJ, et al. Albuminuria is evident in the early stages of diabetes onset: results from the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). American Journal of Kidney Diseases 2004;44(5):792-8.
4. Luk A, Chan JC. Diabetic nephropathy--what are the unmet needs? Diabetes Res clinical practice 2008;82:15-20.
5. Palmer AJ, Valentine WJ, Chen R, Mehin N, Gabriel S, Bregman B ,et al. A health economic analysis of screening and optimal treatment of nephropathy in patients with type 2 diabetes and hypertension in the USA. Nephrology Dialysis Transplantation 2008;23(4):1216-23.
6. Agulló-Ortuño MT, Albaladejo MD, Parra S, Rodríguez-Manotas M, Fenollar M, Ruíz-Espejo F, et al. Plasmatic homocysteine concentration and its relationship with complications associated to diabetes mellitus. Clin Chim Acta. 2002; 326(1-2):105-12.
7. Looker HC, Fagot-Campagna A, Gunter EW, Pfeiffer CM, Narayan KV, Knowler WC, et al. Homocysteine as a risk factor for nephropathy and retinopathy in Type 2 diabetes. Diabetologia 2003;46(6):766-72.
8. Trialists_Collaboration HL. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. BMJ: British Medical Journal 1998;316(7135):894.
9. Buysschaert M, Dramais AS, Wallemacq PE, Hermans MP. Hyperhomocysteinemia in type 2 diabetes: relationship to macroangiopathy, nephropathy, and insulin resistance. Diabetes Care. 2000; 23(12):1816-22.
10. Chico A, Pérez A, Córdoba A, Arcelús R, Carreras G, de Leiva A, Et al. Plasma homocysteine is related to albumin excretion rate in patients with diabetes mellitus: a new link between diabetic nephropathy and cardiovascular disease? Diabetologia. 1998; 41(6):684-93.
11. Lanfredini M, Fiorina P, Peca MG, Veronelli A, Mello A, Astorri E, et al. Fasting and post-methionine load homocyst(e)ine values are correlated with microalbuminuria and could contribute to worsening vascular damage in non-insulin-dependent diabetes mellitus patients. Metabolism. 1998; 47(8):915-21.
12. House AA, Eliasziw M, Cattran DC, Churchill DN, Oliver MJ, Fine A, et al. Effect of B-vitamin therapy on progression of diabetic nephropathy: a randomized controlled trial. JAMA. 2010 28;303(16):1603-9.