Original Article

Korean Diabetes J 2010;34:200-206
doi: 10.4093/kdj.2010.34.3.200
pISSN 1976-9180 · eISSN 2093-2650

kdj
KOREAN DIABETES JOURNAL

Homocysteine as a Risk Factor for Development of Microalbuminuria in Type 2 Diabetes

Eun-Hee Cho*,†, Eun Hee Kim*, Won Gu Kim, Eun Hui Jeong, Eun Hee Koh, Woo-Je Lee, Min-Seon Kim, Joong-Yeol Park, Ki-Up Lee

Division of Endocrinology and Metabolism, Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Background: Kidney function is critical in homocysteine clearance, and plasma homocysteine level is frequently increased in patients with renal failure. On the other hand, recent studies in animals have shown that hyperhomocysteinemia induces renal injury. In this study, we determined whether hyperhomocysteinemia can be a risk factor for the development of microalbuminuria in patients with type 2 diabetes.
Methods: A nested case-control study. Of 887 patients with type 2 diabetes who did not have microalbuminuria at baseline, 76 developed microalbuminuria during follow-up (mean, 36.0 ± 11.7 months; range, 18 to 76 months). The control group consisted of 152 age- and sex-matched subjects who did not develop microalbuminuria. Baseline plasma homocysteine concentrations were measured in stored samples.
Results: Baseline plasma homocysteine concentrations and mean HbA1C levels during follow-up were significantly higher in patients who developed microalbuminuria than in those who remained normoalbuminuric. Multivariate logistic regression analysis showed that baseline plasma homocysteine level and mean HbA1C were independent predictors of microalbuminuria in type 2 diabetes.
Conclusion: Hyperhomocysteinemia was associated with increased risk of microalbuminuria in patients with type 2 diabetes supporting the concept that hyperhomocysteinemia has an etiologic role in the pathogenesis of diabetic nephropathy.

Keywords: Diabetes mellitus; Homocysteine; Microalbuminuria

INTRODUCTION

The public health burden of diabetic nephropathy has increased rapidly along with the world-wide increase in the prevalence of type 2 diabetes. Diabetic nephropathy deteroriates quality of life and also decreases life expectancy. Microalbuminuria is a predictor of overt proteinuria in patients with diabetes [1], as well as being a risk factor for cardiovascular disease in patients with type 2 diabetes and in normal subjects [2]. Although poor glycemic control and high blood pressure are known to increase the risk of microalbuminuria in patients with type 2 diabetes [3-5], little is known regarding other factors that predispose these individuals to the development of microalbuminuria.

Hyperhomocysteinemia occurs frequently in patients with renal failure [6]. As proper renal function is crucial to homocysteine metabolism [7], it is generally believed that hyperhomocysteinemia in renal failure is secondary to decreased renal function. On the other hand, recent studies in animals showed

Corresponding author: Ki-Up Lee
Department of Internal Medicine, University of Ulsan College of Medicine, 388-1 Pungnap2-dong, Songpa-gu, Seoul 138-736, Korea
E-mail: kulee@amc.seoul.kr
*Eun-Hee Cho and Eun Hee Kim jointly contribute to this paper as first authors.
†Current affiliation: Division of Endocrinology and Metabolism, Department of Internal Medicine, Kangwon National University School of Medicine, Chuncheon, Korea
Received: Jun. 7, 2010; Accepted: Jun. 17, 2010

that hyperhomocysteinemia induces glumerulosclerosis and podocyte injury [8-12]. It has also been reported that hyperhomocysteinemia precedes the development of overt proteinuria in patients with type 2 diabetes [13]. These findings suggest that hyperhomocysteinemia may play a pathogenic role in the genesis of diabetic nephropathy. However, it is not yet clear whether hyperhomocysteinemia can be a risk factor for early diabetic nephropathy. We therefore assessed whether hyperhomocysteinemia is an independent risk factor for the development of microalbuminuria in patients with type 2 diabetes.

METHODS

Study protocol

This study was performed in patients with type 2 diabetes attending a diabetes clinic of a university hospital (the Asan Medical Center) in Seoul, South Korea. From January 2002 to December 2004, we recruited 1,226 patients with type 2 diabetes and normoalbuminuria at the time of registry and with urine collection. Patients were excluded if any one of the following conditions was present: plasma creatinine > 1.2 mg/dL, congestive heart failure, or a major concurrent illness such as cancer or end-stage pulmonary or liver disease. After obtaining written informed consent from each individual and the approval of our institutional review committee, we obtained baseline blood samples from each patient, as well as recording patients age, sex, blood pressure, duration of diabetes and other clinical information. Blood samples were centrifuged and stored at -70°C.

At baseline, patients collected timed overnight urine samples 2 or 3 times [5], and their baseline albumin excretion rate was determined by radioimmunoassay (Beckman Coulter, Galway, Ireland). Individuals were considered normoalbuminuric if urinary albumin excretion (UAE) was consistently under 20 μg/min. Patients were followed every 3 months. At each visit, blood pressure, plasma glucose, lipid profiles, and HbA1C were measured. UAE was measured twice yearly from timed overnight urine collections. If the UAE value exceeded 20 μg/min, it was measured twice more at 3-month intervals. Persistent microalbuminuria was defined as UAE > 20 μg/min on at least two of the three measurements.

By June 2007, we collected data on 887 patients who had been followed for more than 18 months (mean follow up, 36.0 ± 11.7 months; range, 18 to 76 months). Among these, 76 subjects developed microalbuminuria during the follow-up period, and we defined them as cases. For each case, we randomly and individually selected two controls who were matched on age and sex.

Laboratory analyses

Fasting plasma glucose, HbA1C, C-peptide, creatinine, high sensitivity C-reactive protein (hsCRP), total cholesterol, triglycerides, high density lipoptoein cholesterol (HDL-C), low density lipoptoein cholesterol (LDL-C) and free fatty acid concentrations were measured by standard procedures. Homocysteine concentrations in the stored plasma samples were measured by competitive immunoassay analyzed on the ADVIA Centaur (Bayer Diagnostics, Tarrytown, NY, USA). Estimated glomerular filtration rate (GFR) was calculated using Cockroft & Gault equation. Matched case-control pairs were handled identically and assayed in random order in the same analytical run.

Statistical analyses

Results are reported as mean ± standard deviation or median with interquartile range. Statistical analysis was performed using SPSS software (version 12.0; SPSS Inc., Chicago, IL, USA). Student's *t*-test or Mann Whitney U test was used for continuous variables, and the χ^2 test for analysis of discrete variables, with all *P* values reported for two-sided tests. Values of $P < 0.05$ were considered significant.

To determine the associations of potential determinants with the development of microalbuminuria, we performed multiple logistic regression analysis with backward elimination of variables, using seven variables that may be of biological significance (i.e., baseline homocysteine and estimated GFR levels, baseline UAE, follow-up duration, mean systolic blood pressure and HbA1C levels during follow-up, past and present cardiovascular disease). The presence of microalbuminuria at follow-up was the dependent variable and potential determinants for development of microalbuminuria were independent variables. Variables having skewed distribution such as baseline plasma homocysteine concentrations, and follow-up duration were natural log (Ln)-transformed before analysis. Values of $P < 0.05$ were considered significant. The dose-response relationship for homocysteine was determined by calculating odds ratios for developing microalbuminuria for several total homocysteine concentration ranges (9.1 to 14.0, 14.1 to 19.0, and > 19.0 μmol/L) with total homocysteine concentrations < 9.1 μmol/L as reference [14].

Table 1. Clinical characteristics at baseline relative to albuminuria status at follow-up

	Patients who did not develop microalbuminuria	Patients who developed microalbuminuria	*P* value[a]
n (M/F)	152 (106/46)	76 (53/23)	0.762
Age, yr	57 (48 to 65)	57 (48 to 65)	0.821
BMI, kg/m^2	25.2 ± 3.2	25.4 ± 2.8	0.722
Duration of diabetes, yr	5 (3 to 10)	6 (4 to 10)	0.804
Fasting plasma glucose, mg/dL	153.0 ± 44.9	146.6 ± 41.5	0.295
HA1C, %	8.1 ± 1.7	8.3 ± 1.9	0.274
Systolic blood pressure, mm Hg	134.4 ± 16.6	135.0 ± 14.7	0.801
Diastolic blood pressure, mm Hg	79.4 ± 10.7	78.6 ± 9.3	0.588
Total cholesterol, mg/dL	189.4 ± 36.8	189.8 ± 41.3	0.939
C-peptide, nmol/L	1.8 (1.3 to 2.2)	1.8 (1.3 to 2.4)	0.999
Creatinine, mg/dL	0.88 ± 0.17	0.92 ± 0.21	0.489
eGFR, mL/min/1.73m^2	92.6 ± 23.6	94.1 ± 30.9	0.739
hsCRP, mg/dL	0.13 (0.08 to 0.25)	0.12 (0.08 to 0.2)	0.568
Free fatty acid, μEq/L	680 (516 to 954)	746 (530 to 977)	0.290
Homocysteine, μmol/L	10.3 ± 3.1	11.8 ± 3.7	0.003
Baseline urinary albumin excretion, μg/min	10.6 (7.9 to 14.2)	11.4 (8.0 to 16.1)	0.369
Baseline hypertension prevalence	59 (38.8)	33 (43.4)	0.567
Baseline ARB or ACEi medication	36 (23.7)	27 (35.5)	0.083
Baseline statin medication	47 (30.9)	25 (32.9)	0.765
Baseline anti-diabetic therapy			
Sulfonylurea	124 (81.6)	61 (80.3)	0.624
Metformin	93 (61.2)	45 (59.2)	0.235
Other hypoglycemic agents	61 (40.1)	32 (47.8)	0.542
Insulin alone	12 (7.9)	9 (11.8)	0.375
Insulin + oral agents	25 (16.4)	10 (13.2)	0.440
Past and present CVD history	22 (14.5)	14 (18.4)	0.443

Data are means ± standard deviation or median (interquartile range), *n* (%).
BMI, body mass index; ARB, angiotensin receptor blockers; ACEi, angiotensin converting enzyme inhibitors; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease.
[a]Appropriate bivariate statistic.

RESULTS

Table 1 shows the baseline clinical characteristics of patients who did and did not develop microalbuminuria. We observed no significant between-group differences in baseline age, sex, body mass index (BMI), duration of diabetes, fasting plasma glucose, systolic and diastolic blood pressure, UAE, and HbA1C, total cholesterol, C-peptide, creatinine, estimated GFR, hsCRP, free fatty acid concentrations, prevalence of hypertension, the use of antihypertensive or anti-diabetic medications and past and present cardiovascular disease history. There was no statistically significant correlation between Ln plasma homocysteine level and baseline creatinine levels or UAE (Fig. 1A and B).

Baseline concentrations of plasma homocysteine, however, were significantly higher among patients who developed microalbuminuria than among patients who were normoalbuminuric during the follow-up period (11.8 ± 3.7 μmol/L vs. 10.3 ± 3.1 μmol/L, $P = 0.003$). There was a significant positive correlation between baseline Ln plasma homocysteine levels

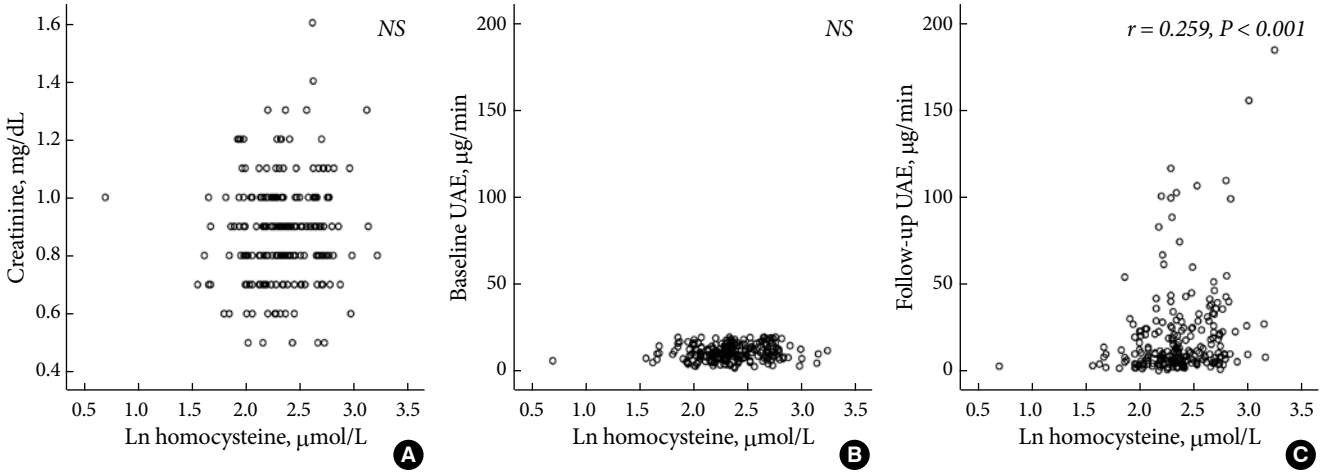

Fig. 1. Correlations between (A) basal plasma creatinine levels or (B) baseline urinary albumin excretion rate and Ln plasma homocysteine level, and between (C) follow-up urinary albumin excretion rate and Ln plasma homocysteine level among the 76 cases and 152 control subjects. UAE, urinary albumin excretion.

Table 2. Clinical characteristics during follow-up period relative to albuminuria status at follow-up

	Patients who did not develop microalbuminuria	Patients who developed microalbuminuria	*P* value[a]
n (M/F)	152 (106/46)	76 (53/23)	0.762
Mean Fasting plasma glucose, mg/dL	141.0 ± 29.8	149.1 ± 34.7	0.071
Mean HbA1C, %	7.5 ± 0.9	8.0 ± 1.2	0.001
Mean systolic blood pressure, mm Hg	134.2 ± 16.2	135.6 ± 15.6	0.651
Mean diastolic blood pressure, mm Hg	76.2 ± 8.4	76.2 ± 7.2	0.955
Mean total cholesterol, mg/dL	179.2 ± 26.0	183.4 ± 26.0	0.249
Follow up duration, mon	31.5 (29 to 40)	30.5 (27 to 39)	0.192

[a]Appropriate bivariate statistic.

Table 3. Multiple logistic regression analysis with the background elimination method using 6 biologically important variables as independent variables, and the development of microalbuminuria as the dependent variable

Independent variables	Partial regression coefficient	Standard error	Odds ratio (β)	95 % CI	*P* value
Ln homocysteine	1.642	0.511	5.167	1.898 to 14.067	0.001
Mean HbA1C	0.560	0.153	1.750	1.297 to 2.362	<0.001

The multivariate model was adjusted for baseline estimated glomerular filtration rate, baseline urinary albumin excretion, mean systolic blood pressure, and follow-up duration and past and previous cardiovascular disease history. Natural log transformed (Ln) data were modeled for creatinine, homocysteine and follow-up duration. CI, confidence interval.

and follow-up UAE (Fig. 1C).

There were no significant between group differences in duration of follow up, mean systolic blood pressure, mean diastolic blood pressure, and mean total cholesterol (Table 2). However, mean HbA1C during follow-up were significantly higher among patients who developed microalbuminuria than among patients who remained normoalbuminuric (7.5 ± 0.9% vs. 8.0 ± 1.2%, $P = 0.001$).

Multivariate binary logistic regression analysis with backward elimination of variables was performed using baseline Ln plasma homocysteine and estimated GFR levels, baseline UAE, Ln follow-up duration, mean systolic blood pressure and

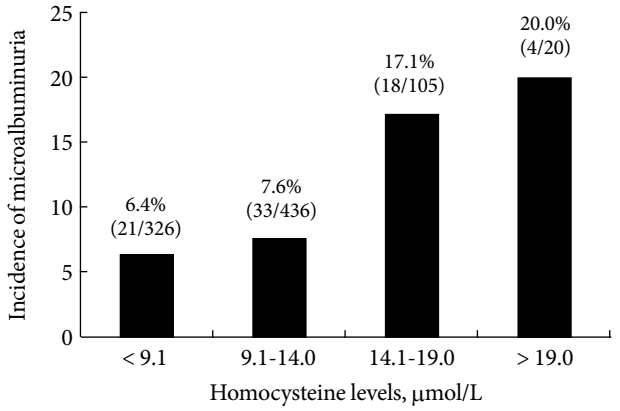

Fig. 2. Cumulative incidence of microalbuminuria per category of homocysteine level. *P* for trend was 0.001.

mean HbA1C levels during follow-up, and past and present cardiovascular disease history as independent variables, and microalbuminuria development as the dependent variable. Mean HbA1C (odds ratio [OR], 1.750; range, 1.297 to 2.362; $P < 0.001$) and Ln homocysteine levels (OR, 5.167; range, 1.898 to 14.067; $P = 0.001$) were independent predictors of microalbuminuria development (Table 3).

We divided the total subjects in whom baseline total homocysteine concentrations were measured ($n = 887$) into those with baseline homocysteine concentrations of < 9.1, 9.1 to 14.0, 14.1 to 19.0, and > 19.0 μmol/L. The cumulative incidence of microalbuminuria in these 4 groups of patients was 6.4% (21/326), 7.6% (33/436), 17.1% (18/105), and 20.0% (4/20), respectively (*P* value for trend = 0.001, Fig. 2). After adjustment for age, sex, baseline HbA1C and plasma creatinine concentrations, the odds ratios for microalbuminuria, relative to baseline homocysteine concentrations of < 9.1 μmol/L, were 1.3 (95% confidence interval [CI], 0.7 to 2.4), 3.6 (95% CI, 1.8 to 7.3), and 4.6 (95% CI, 1.4 to 15.4) for baseline homocysteine concentrations of 9.1 to 14.0, 14.1 to 19.0, and > 19.0 μmol/L, respectively. When homocysteine level categories were replaced by homocysteine concentration as a continuous variable, a 5 μmol/L increase in homocysteine concentration was associated with a 1.8-fold (95% CI, 1.3 to 2.5; $P < 0.001$) increase in the risk of developing microalbuminuria, after adjustment for age, sex, baseline HbA1c and creatinine levels.

DISCUSSION

In this study, we show that hyperhomocysteinemia is an independent risk factor for the development of microalbuminuria in patients with type 2 diabetes. Baseline plasma homocysteine concentrations were significantly higher in normoalbuminuric patients who developed microalbuminuria during follow-up than in normoalbuminuric patients who did not develop microalbuminuria. These findings are in agreement with previous cross-sectional and longitudinal studies [13,15], which showed associations between plasma homocysteine levels and overt proteinuria in diabetic patients. Although homocysteine concentrations have been found to be associated with microalbuminuria development in non-diabetic subjects [14], to our knowledge, the present study is the first to show an association between plasma homocysteine concentration and development of microalbuminuria in diabetic individuals. Although the previous study by Jager et al. was unable to find a relationship between hyperhomocysteinemia and microalbuminuria developement in diabetic patients [14], our results showed the significant relationship in patients with type 2 diabetes because of a larger sample size.

Kidney function is critical for homocysteine clearance [7], and hyperhomocysteinemia occurs frequently in patients with renal failure [6,16]. Thus it can be asked whether the association between plasma homocysteine levels and urinary albumin excretion is attributed to associated changes in renal function [16,17]. In our study, however, only patients who were normoalbuminuric at baseline were included. Although we did not measure glomerular filtration rate in all of these patients, renal function is known to deteriorate following (micro)albuminuria [18,19]. All of our subjects had normal plasma creatinine levels (below 1.2 mg/dL), and there was no significant correlation between baseline Ln plasma homocysteine level and baseline UAE or baseline plasma creatinine concentration. On the other hand, follow-up UAE level was significantly correlated with baseline Ln plasma homocysteine concentration.

Homocysteine has been shown to cause vascular disease and endothelial damage [20-22]. In addition, recent studies suggested that homocysteine is toxic to kidney tissues. Diet-induced chronic hyperhomocysteinemia could induce arterial and arteriolar thickening, and tubulointerstitial and podocyte injury in the kidney [8,9]. Regarding the mechanism of homocysteine-induced glomerular injury, homocysteine was shown to activate MAP kinases and to induce endoplasmic reticulum stress in cultured mesangial cells [10]. It was also shown that homocysteine stimulates ceramide-mediated redox signaling [11], and increases monocyte chemoattractant protein-1 ex-

pression in the kidney via nuclear factor-kappaB activation [12]. These studies suggest that hyperhomocysteinemia may play a causative role in early renal injury, in addition to being a marker of impaired renal function [16,17].

Poor glycemic control and high blood pressure are known to increase the risk of microalbuminuria in patients with type 2 diabetes [3-5]. As expected, we found significant differences between cases and controls in mean HbA1C levels during follow-up. In addition, multivariate logistic regression analyses showed that basal Ln plasma homocysteine levels remained a significant independent variable, even after correcting for mean HbA1C levels and other important variables. We also found that plasma homocysteine concentration > 14.0 μmol/L was associated with a marked increase in the risk of microalbuminuria. For each 5 μmol/L increase in homocysteine concentration, the risk of developing microalbuminuria increased by about 80%. This is in line with previous studies showing 1.3 to 1.6 fold increase per 5 μmol/L increase in homocysteine concentration in the risk for microalbuminuria in non-diabetic subjects [23] and of overt proteinuria in diabetic subjects [13].

Although microalbuminuria in type 1 diabetes is regarded as an early manifestation of diabetic nephropathy, the meaning of microalbuminuria in type 2 diabetes is more complex. The metabolic syndrome and obesity, which frequently accompany type 2 diabetes, predispose these individuals to microalbuminuria [24], and plasma homocysteine level is frequently increased in patients with metabolic syndrome [25,26].

In the present study, 125 among 887 patients had marked hyperhomocysteinemia (> 14 μmol/L) and increased risk for developing microalbuminuria. Since homocysteine has been shown to cause cardiovascular disease and endothelial damage [20-22], hyperhomocysteinemia in diabetic patients may lead to endothelial dysfunction in systemic and renal blood vessels, and predispose these patients to increased risks of both cardiovascular disease and microalbuminuria.

In our study, we used nested cohort design with a relatively small number of patients attending a single center. For many research questions, the nested case-control design potentially gives significant reductions in time, cost, and effort of data collection and analysis, compared with the full cohort approach, with a relatively minor loss in statistical efficiency [27,28]. However, future studies with a larger population and longer duration of follow-up are needed to provide more definitive conclusion.

In summary, we have shown that hyperhomocysteinemia is associated with a higher incidence of microalbuminuria in patients with type 2 diabetes mellitus. Further studies in larger populations are needed to confirm these findings, and to test whether lowering of plasma homocysteine by dietary manipulation [29] is helpful for patients at risk for diabetic nephropathy.

ACKNOWLEDGEMENT

Support: This work was supported by the Korea Science and Engineering Foundation (KOSEF) grants funded by the Ministry of Science and Technology (M10642140004-06N4214-00410: K.U.L. and M10753020003-08N5302-00310: J.Y.P. and NRL M1040000000804J000000810: J.Y.P.) and a grant (2008-122) from Asan Institute for Life Sciences, Seoul, Korea.
Conflict of interest: None.

REFERENCES

1. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. N Engl J Med 1984;310:356-60.
2. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, Halle JP, Young J, Rashkow A, Joyce C, Nawaz S, Yusuf S. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. JAMA 2001;286:421-6.
3. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000;321:405-12.
4. Gall MA, Hougaard P, Borch-Johnsen K, Parving HH. Risk factors for development of incipient and overt diabetic nephropathy in patients with non-insulin dependent diabetes mellitus: prospective, observational study. BMJ 1997;314:783-8.
5. Park JY, Kim HK, Chung YE, Kim SW, Hong SK, Lee KU. Incidence and determinants of microalbuminuria in Koreans with type 2 diabetes. Diabetes Care 1998;21:530-4.
6. Hoffer LJ. Testing the homocysteine hypothesis in end-stage renal disease: problems and a possible solution. Kidney Int 2006;69:1507-10.
7. Ruan L, Chen W, Srinivasan SR, Xu J, Toprak A, Berenson GS. Plasma homocysteine is adversely associated with glomerular

filtration rate in asymptomatic black and white young adults: the Bogalusa heart study. Eur J Epidemiol 2009;24:315-9.
8. Kumagai H, Katoh S, Hirosawa K, Kimura M, Hishida A, Ikegaya N. Renal tubulointerstitial injury in weanling rats with hyperhomocysteinemia. Kidney Int 2002;62:1219-28.
9. Yi F, dos Santos EA, Xia M, Chen QZ, Li PL, Li N. Podocyte injury and glomerulosclerosis in hyperhomocysteinemic rats. Am J Nephrol 2007;27:262-8.
10. Ingram AJ, Krepinsky JC, James L, Austin RC, Tang D, Salapatek AM, Thai K, Scholey JW. Activation of mesangial cell mapk in response to homocysteine. Kidney Int 2004;66:733-45.
11. Yi F, Zhang AY, Li N, Muh RW, Fillet M, Renert AF, Li PL. Inhibition of ceramide-redox signaling pathway blocks glomerular injury in hyperhomocysteinemic rats. Kidney Int 2006;70: 88-96.
12. Hwang SY, Woo CW, Au-Yeung KK, Siow YL, Zhu TY, O K. Homocysteine stimulates monocyte chemoattractant protein-1 expression in the kidney via nuclear factor-kappaB activation. Am J Physiol Renal Physiol 2008;294:F236-44.
13. Looker HC, Fagot-Campagna A, Gunter EW, Pfeiffer CM, Narayan KM, Knowler WC, Hanson RL. Homocysteine as a risk factor for nephropathy and retinopathy in type 2 diabetes. Diabetologia 2003;46:766-72.
14. Jager A, Kostense PJ, Nijpels G, Dekker JM, Heine RJ, Bouter LM, Donker AJ, Stehouwer CD. Serum homocysteine levels are associated with the development of (micro)albuminuria: The Hoorn Study. Arterioscler Thromb Vasc Biol 2001;21:74-81.
15. Chico A, Perez A, Cordoba A, Arcelus R, Carreras G, de Leiva A, Gonzalez-Sastre F, Blanco-Vaca F. Plasma homocysteine is related to albumin excretion rate in patients with diabetes mellitus: a new link between diabetic nephropathy and cardiovascular disease? Diabetologia 1998;41:684-93.
16. Wollesen F, Brattstrom L, Refsum H, Ueland PM, Berglund L, Berne C. Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. Kidney Int 1999; 55:1028-35.
17. Davies L, Wilmshurst EG, McElduff A, Gunton J, Clifton-Bligh P, Fulcher GR. The relationship among homocysteine, creatinine clearance, and albuminuria in patients with type 2 diabetes. Diabetes Care 2001;24:1805-9.
18. Perkins BA, Ficociello LH, Ostrander BE, Silva KH, Weinberg J, Warram JH, Krolewski AS. Microalbuminuria and the risk for early progressive renal function decline in type 1 diabetes. J Am Soc Nephrol 2007;18:1353-61.
19. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney Int 2003;63:225-32.
20. Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. J Clin Invest 1986;77:1370-6.
21. Woo KS, Chook P, Lolin YI, Cheung AS, Chan LT, Sun YY, Sanderson JE, Metreweli C, Celermajer DS. Hyperhomocyst(e) inemia is a risk factor for arterial endothelial dysfunction in humans. Circulation 1997;96:2542-4.
22. Castro R, Rivera I, Blom HJ, Jakobs C, Tavares de Almeida I. Homocysteine metabolism, hyperhomocysteinaemia and vascular disease: an overview. J Inherit Metab Dis 2006;29:3-20.
23. Hoogeveen EK, Kostense PJ, Jager A, Heine RJ, Jakobs C, Bouter LM, Donker AJ, Stehouwer CD. Serum homocysteine level and protein intake are related to risk of microalbuminuria: the Hoorn Study. Kidney Int 1998;54:203-9.
24. Locatelli F, Pozzoni P, Del Vecchio L. Renal manifestations in the metabolic syndrome. J Am Soc Nephrol 2006;17:S81-5.
25. Lee KU. Oxidative stress markers in Korean subjects with insulin resistance syndrome. Diabetes Res Clin Pract 2001;54 Suppl 2:S29-33.
26. Bjorck J, Hellgren M, Rastam L, Lindblad U. Associations between serum insulin and homocysteine in a Swedish population-a potential link between the metabolic syndrome and hyperhomocysteinemia: the Skaraborg project. Metabolism 2006; 55:1007-13.
27. Ernster VL. Nested case-control studies. Prev Med 1994;23: 587-90.
28. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, Curhan GC, Rifai N, Cannuscio CC, Stampfer MJ, Rimm EB. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med 2004;351:2599-610.
29. Clarke R, Refsum H, Birks J, Evans JG, Johnston C, Sherliker P, Ueland PM, Schneede J, McPartlin J, Nexo E, Scott JM. Screening for vitamin B-12 and folate deficiency in older persons. Am J Clin Nutr 2003;77:1241-7.