CLINICAL SCIENCE

Ischemia-modified albumin in type 2 diabetic patients with and without peripheral arterial disease

Shao-gang MA, Chun-ling WEI, Bing HONG, Wei-nan YU

Department of Endocrinology and Metabolism, the Affiliated Huai'an Hospital of Xuzhou Medical College, Huai'an, China.

OBJECTIVE: To determine whether there is an association between serum ischemia-modified albumin and the risk factor profile in type 2 diabetic patients with peripheral arterial disease and to identify the risk markers for peripheral arterial disease.

METHODS: Participants included 290 patients (35.2% women) with type 2 diabetes. The ankle-brachial pressure index was measured using a standard protocol, and peripheral arterial disease was defined as an ankle-brachial index <0.90 or ≥ 1.3. The basal ischemia-modified albumin levels and clinical parameters were measured and analyzed. The risk factors for peripheral arterial disease were examined by multiple logistic analyses.

RESULTS: Age, systolic blood pressure, total cholesterol, low-density lipoprotein cholesterol, urine albumin, homocysteine, and ischemia-modified albumin were significantly higher in patients with peripheral arterial disease than in disease-free patients ($p<0.05$), while ankle-brachial index was lower in the former group ($p<0.05$). Ischemia-modified albumin was positively associated with HbA1c and homocysteine levels ($r = 0.220$, $p = 0.030$; $r = 0.446$, $p = 0.044$, respectively), while no correlation was found with ankle-brachial index. Multiple logistic analyses indicated that HbA1c, systolic blood pressure, homocysteine and ischemia-modified albumin were independent risk factors for peripheral arterial disease in the diabetic subjects.

CONCLUSION: The baseline ischemia-modified albumin levels were significantly higher and positively associated with HbA1c and homocysteine levels in type 2 diabetic patients with peripheral arterial disease. Ischemia-modified albumin was a risk marker for peripheral arterial disease. Taken together, these results might be helpful for monitoring diabetic peripheral arterial disease.

KEYWORDS: Diabetes mellitus; Peripheral arterial disease; Ischemia-modified albumin; Risk factors; Biomarker.

Shao-gang MA, Chun-ling WEI, Bing HONG, Wei-nan YU. Ischemia-modified albumin in type 2 diabetic patients with and without peripheral arterial disease. Clinics. 2011;66(10):1677-1680.

Received for publication on March 22, 2011; **First review completed on** May 9, 2011; **Accepted for publication on** June 14, 2011

E-mail: mashaogang@163.com

Tel.: 86 517 80871820

INTRODUCTION

Peripheral arterial disease (PAD) is a clinical manifestation of atherosclerotic arterial occlusive disease that affects the lower extremities. It is also one of the most severe complications of type 2 diabetes mellitus (T2DM) and a major cause of morbidity and mortality; patients with PAD have an increased risk of vascular events.[1,2] Peripheral arterial disease affects 8 to 12 million individuals in the United States and is also prevalent in Europe and Asia.[3-5] A cluster of conditions that are associated with metabolic abnormality, such as smoking, advanced age, hypertension, dyslipidemia, and high levels of homocysteine, are important risk factors for PAD. More than 95% of persons with PAD have one or more cardiovascular disease risk factors.[6-8]

In PAD, biomarkers are released into the circulation from lower extremity arterial beds vascular endothelium. A blood test for PAD that measures these biomarkers, if sufficiently sensitive and specific, would be expected to improve recognition and treatment of affected individuals. Biomarkers for PAD also might be useful in determining prognosis, the risk for progression. Both traditional and nontraditional risk biomarkers are independently associated with PAD,[9] and monitoring nontraditional risk biomarkers of PAD in diabetes might be particularly important. Among persons with PAD, circulating levels of D-dimer is higher 1 to 2 years before death than in periods more remote from death. D-dimer levels increase is independently associated with higher mortality in persons with PAD.[10] In comparison to D-dimer, ischemia-modified albumin is a relatively new and sensitive biomarker for evaluating patients with ischemic events, especially for the early diagnosis of myocardial ischemia.[11,12] Ischemia-modified albumin, also called cobalt-binding albumin, is produced as a result of serum albumin flowing through ischemic tissues and is a marker of oxidative stress and ischemia, as serum levels of modified albumin rise in many diseases accompanied by ischemia.

No potential conflict of interest was reported.

Whether the modified albumin is associated with systemic atherosclerotic burden is not known. Until now, few studies in the literature have evaluated ischemia-modified albumin levels in patients with PAD. We hypothesized that the modified albumin levels might be affected by ischemia occurring in PAD and that modified albumin levels could be useful for the evaluation of hypoxia associated with a low ankle-brachial pressure index (ABI; ratio of ankle blood pressure to brachial blood pressure). The ABI is a simple and reliable means of diagnosing PAD. In this study, we investigated whether serum ischemia-modified albumin is associated with the ABI and/or with atherosclerotic risk factors in T2DM. A secondary objective was to identify whether serum ischemia-modified albumin is a nontraditional risk marker for PAD.

METHODS

Patients

This study was performed in the department of endocrinology of the Affiliated Huai'an Hospital of Xuzhou Medical College in Huai'an, P.R. China. All participants gave informed consent. The study was approved by the Ethics Committee of the hospital, in accordance with the Declaration of Helsinki. The study included 290 patients (35.2% women) with T2DM, 110 of whom had coexisting PAD. The diagnoses of PAD were confirmed by color Doppler ultrasonography, a diagnosis of PAD was based on an ABI <0.9 or ≥ 1.3 in either leg, as described in previous studies.[3-5]

The PAD group consisted of 68 males and 42 females between 52 and 78 years old. The non-PAD group consisted of 120 males and 60 females between 50 and 77 years old. In addition to age and gender, for each patient we recorded the blood pressure, diabetes and hypertension duration and other clinical information. All selected patients were without liver and kidney dysfunction, ischemic events, infection, and corticosteroid therapy.

Measurements

Blood samples were drawn from all subjects into vacutainer tubes by venipuncture, centrifuged and stored at -20℃ for a maximum of four weeks before measurement of ischemia-modified albumin and homocysteine levels. The level of modified albumin was measured as previously described,[13-15] and the results were reported in absorbance units. For the applied technique of manual ischemia-modified albumin determination, the intra- and inter-assay coefficient of variations (CV) were 2.37% and 4.12%, respectively.

The homocysteine concentration was measured by a competitive immunoassay kit (Changsha Yikang Institute of Biological Technology, Changsha, China). The intra- and inter-assay CVs were 2.68% and 3.32%, respectively. Urine albumin was measured using a radioimmunoassay kit (Beijing North Institute of Biological Technology, Beijing, China). The intra- and inter-assay CVs were 3.37% and 3.07%, respectively.

The HbA1c level was measured on a Bio-Rad Laboratories Ltd. (Shanghai, China) HbA1c meter. The final HbA1c test result was calculated from the HbA1c/Hb ratio.[16] The intra- and inter-assay precisions were CV 2.4% and CV 1.7%, respectively, and the sensitivity was 0.68 μmol/l for hemoglobin and 0.71 μmol/l for HbA1c. Plasma glucose, creatinine, uric acid, total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were measured by standard procedures.

Statistical Analyses

Statistical analyses were conducted with Statistical Package for the Social Sciences 11.0 for Windows (SPSS Inc, Chicago, IL). All quantitative data were expressed as the mean $\pm$standard deviation ($\bar{x} \pm$ SD). Differences between groups were analyzed by unpaired Student's t-tests and Chi-squared tests. Linear correlation coefficients between the modified albumin and various parameters were calculated. The risk factors for PAD were examined by multiple logistic analyses. A two-tailed $p<0.05$ was considered statistically significant.

RESULTS

The clinical results of the survey of the PAD and non-PAD groups are shown in Table 1. Indexes in the PAD group, including age, HbA1c, systolic blood pressure, low-density lipoprotein cholesterol, total cholesterol, urine albumin, homocysteine, and ischemia-modified albumin, were higher than those of the non-PAD group ($p<0.05$). ABI was lower ($p = 0.023$). There were no significant differences between the two groups with respect to gender, diastolic blood pressure, high-density lipoprotein cholesterol, triglycerides, and serum uric acid.

Table 2 shows the correlation between ischemia-modified albumin levels and age, ABI, and other variables in both groups. Modified albumin was positively associated with HbA1c and homocysteine ($r = 0.220$, $p = 0.030$; $r = 0.446$, $p = 0.044$, respectively), while no correlation was found with ABI or other variables.

To examine the risk factors to PAD, a multiple logistic regression analysis with the eight significant variables (age, HbA1c, systolic blood pressure, low-density lipoprotein cholesterol, total cholesterol, urine albumin, homocysteine, and ischemia-modified albumin) was performed. The results are shown in Table 3. The odds ratios and 95% confidence intervals for PAD were calculated. The levels of HbA1c, systolic blood pressure, homocysteine, and modified albumin were independent risk factors to PAD ($p<0.05$).

DISCUSSION

Modified albumin is also a novel indicator of widespread endothelial damage and ischemia in diabetic patients.[17] In this study, ischemia-modified albumin levels were measured, and correlations between the modified albumin and HbA1c and homocysteine were described. Subjects with PAD had higher modified albumin levels and lower ABIs than patients without PAD. The increase in modified albumin and ABI could indicate an important clinical anoxic condition.

Recent studies demonstrate that the atherosclerotic process in the lower extremities in PAD is not confined to conduit vessels but also affects skeletal muscle flow reserve, metabolism and endothelial cells.[9] Microvascular endothelial activity and skeletal muscle microvascular flow are significantly reduced in both the upper and lower extremities of PAD patients.[18,19] The biological explanation of this association between PAD and ischemia-modified

Table 1 - Clinical parameters of non-PAD and PAD groups with T2DM.

Parameters	non-PAD group (N = 180) Mean ± SD	PAD group (N = 110) Mean ± SD	p-value
Gender (women/men)	60/120	42/68	0.157
Age (years)	55.2±9.2	62.1±7.9	**0.015**
Ankle-brachial index	1.1±0.12	0.69±0.11	**0.023**
Systolic blood pressure (mmHg)	126.7±11.0	139.2±13.6	**0.033**
Diastolic blood pressure (mmHg)	82.8±7.5	89.2±8.0	0.702
HbA1c (%)	7.6±0.3	9.1±0.3	**0.043**
Serum creatinine (μmol/L)	85.9±6.1	92.8±8.2	0.064
Serum uric acid (μmol/L)	311.5±93.0	341.7±112.0	0.657
Urine albumin (mg/L)	23.1±10.0	120.1±21.1	**0.032**
Serum albumin (g/L)	45.1±1.1	44.2±1.2	0.171
Total cholesterol (mmol/L)	4.6±1.4	5.7±1.2	**0.035**
Triglycerides (mmol/L)	2.0±1.2	2.6±1.1	0.422
High-density lipoprotein cholesterol (mmol/L)	1.4±0.3	1.1±0.3	0.446
Low-density lipoprotein cholesterol (mmol/L)	2.9±0.8	4.3±1.0	**0.036**
Homocysteine (μmol/L)	7.4±1.3	11.8±1.0	**0.025**
Ischemia-modified albumin (U/L)	0.351±0.13	0.841±0.25	**0.039**

albumin is related to a decrease in lower extremity perfusion and oxygenation, thus triggering albumin modification. High plasma modified albumin levels in diabetic subjects might indicate subclinical vascular disease. Microvascular dysfunction is a potential pathophysiological mechanism of PAD in such cases.

There have been several studies on the modified albumin in T2DM, but none considered PAD.[20-22] These studies suggest that the ischemia-albumin molecule in the plasma of diabetic patients is modified by the chronic hypoxic conditions provoked mainly by hyperglycemia and oxidative stress. The HbA1c in T2DM is closely associated with complications, including PAD; higher HbA1c levels reflect a higher prevalence of severe PAD.[23] Serum modified albumin in the PAD group was higher than in the non-PAD group. There was a significant correlation between modified albumin and HbA1c. When HbA1c increases and the ABI decreases, the modified albumin might increase at the same time. The elevated modified albumin levels could be related to the severity of PAD. Levels of HbA1c and ischemia-modified albumin might be biomarkers of developing PAD. Long-term testing and maintenance of HbA1c and blood pressure in the normal range are of great importance for the prevention and delay of vascular complications of T2DM.

Levels of modified albumin were positively correlated with HbA1c and homocysteine, while no correlative relationship was found with ABI. As a biochemical marker, ischemia-modified albumin could be influenced by metabolic factors. The finding of a lack of a correlation with ABI needs further investigation. The PAD group, which had higher modified albumin levels, also presented higher levels of atherosclerotic risk factors, such as poor long-term glycemic control, hypertension, homocysteine, and dyslipidemia. The duration of diabetes, aging, hypertension, dyslipidemia, and advanced hemoglobin glycosylation are known risk factors of PAD.[6-8]

It has also been shown that homocysteine can cause endothelial cell damage and vascular disease; Homocysteine is considered a major independent risk factor for coronary artery disease, stroke, and PAD. An elevated plasma homocysteine level (>15 μmoL) was associated with an increased risk of PAD in a meta-analysis of 27 studies.[24] Higher homocysteine levels were associated with a lower ABI, independent of conventional risk factors.[25] Few studies to date have investigated the association of homocysteine levels with ischemia-modified albumin in PAD. We report here that homocysteine levels are positively correlated with ischemia-modified albumin, such that high amounts of modified albumin might be accompanied by high homocysteine levels.

Table 2 - Correlations between the IMA and clinical parameters in the diabetic subjects.

Parameters	non-PAD group		PAD group	
	r	p-value	r	p-value
Age (years)	0.001	0.985	0.006	0.965
Ankle-brachial index	-0.012	0.405	-0.022	0.315
Systolic blood pressure (mmHg)	0.063	0.834	0.051	0.716
Diastolic blood pressure (mmHg)	0.121	0.092	0.180	0.192
HbA1c (%)	0.082	0.081	0.220	**0.030**
Serum creatinine (μmol/L)	0.056	0.650	0.158	0.151
Serum uric acid (μmol/L)	0.118	0.227	0.208	0.127
Urine albumin (mg/L)	0.065	0.893	0.459	0.696
Serum albumin (g/L)	0.085	0.562	0.125	0.365
Total cholesterol (mmol/L)	0.130	0.703	0.031	0.823
Triglycerides (mmol/L)	0.067	0.128	0.165	0.229
High-density lipoprotein cholesterol (mmol/L)	0.013	0.340	0.053	0.701
Low-density lipoprotein cholesterol (mmol/L)	0.134	0.280	0.037	0.786
Homocysteine (μmol/L)	0.247	0.108	0.446	**0.044**

Table 3 - Multiple logistic regression analysis using PAD as a dependent variable with all subjects (n = 290).

Parameters	Multiple logistic regression analysis			
	Wald	OR	p-value	95% CI
Age (years)	0.725	0.640	0.502	0.790 - 1.012
Systolic blood pressure (mmHg)	4.850	1.751	**0.023**	1.517 - 3.437
HbA1c (%)	3.801	1.682	**0.034**	1.867 - 3.640
Urine albumin (mg/L)	1.471	1.002	0.093	0.968 - 1.168
Total cholesterol (mmol/L)	1.907	1.196	0.068	1.714 - 2.223
Low-density lipoprotein cholesterol (mmol/L)	1.273	0.915	0.541	0.918 - 1.515
Homocysteine (μmol/L)	3.084	1.250	**0.045**	1.016 - 2.215
Ischemia-modified albumin (U/L)	3.455	1.516	**0.040**	1.216 - 2.368

The biomarkers modified albumin could also help to identify individuals with complex metabolic conditions who have a higher risk of suffering from PAD.

CONCLUSION

Measuring the biomarkers ischemia-modified albumin and homocystein, combined with using the ankle-brachial index, could be helpful in monitoring and early diagnosis peripheral arterial disease in T2DM.

REFERENCES

1. Daskalopoulou SS, Daskalopoulos ME, Liapis CD, Mikhailidis DP. Peripheral arterial disease: a missed opportunity to administer statins so as to reduce cardiac morbidity and mortality. Curr Med Chem. 2005;12:443–52.
2. Hankey GJ, Norman PE, Eikelboom JW. Medical treatment of peripheral arterial disease. JAMA. 2006;295:547–53, doi: 10.1001/jama.295.5.547.
3. Hiatt WR, Hoag S, Hamman RF. Effect of diagnostic criteria on the prevalence of peripheral arterial disease. The San Luis Valley Diabetes Study. Circulation. 1995;91:1472–9.
4. Criqui MH. Peripheral arterial disease-epidemiological aspects. Vasc Med. 2001;6(3 Suppl):3–7, doi: 10.1177/1358836X0100600i102.
5. Li J, Luo Y, Xu Y, Yang J, Zheng L, Hasimu B, et al. Risk factors of peripheral arterial disease and relationship between low ankle-brachial index and mortality from all-cause and cardiovascular disease in Chinese patients with type 2 diabetes. Circ J. 2007;71:377–81, doi: 10.1253/circj.71.377.
6. Tapp RJ, Balkau B, Shaw JE, Valensi P, Cailleau M, Eschwege E. Association of glucose metabolism, smoking and cardiovascular risk factors with incident peripheral arterial disease: the DESIR study. Atherosclerosis. 2007;190:84–9, doi: 10.1016/j.atherosclerosis.2006.02.017.
7. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. JAMA. 2001;285:2481–5, doi: 10.1001/jama.285.19.2481.
8. Zanati SG, Mouraria GG, Matsubara LS, Giannini M, Matsubara BB. Profile of cardiovascular risk factors and mortality in patients with symptomatic peripheral arterial disease. Clinics. 2009;64:323–6, doi: 10.1590/S1807-59322009000400010
9. Cooke JP, Wilson AM. Biomarkers of peripheral arterial disease. J Am Coll Cardiol. 2010;55:2017–23, doi: 10.1016/j.jacc.2009.08.090.
10. Vidula H, Tian L, Liu K, Criqui MH, Ferrucci L, Pearce WH, et al. Biomarkers of inflammation and thrombosis as predictors of near-term mortality in patients with peripheral arterial disease: a cohort study. Ann Intern Med. 2008;148:85–93.
11. da Silva SH, Hausen Bdos S, da Silva DB, Becker AM, de Campos MM, Duarte MM, et al. Characteristics of a nickel-albumin binding assay for assessment of myocardial ischaemia. Biomarkers. 2010;15:353–7, doi: 10.3109/13547501003763369.
12. Van Belle E, Dallongeville J, Vicaut E, Degrandsart A, Baulac C, Montalescot G, et al. Ischemia-modified albumin levels predict long-term outcome in patients with acute myocardial infarction. The French Nationwide OPERA study. Am Heart J. 2010;159:570–6, doi: 10.1016/j.ahj.2009.12.026.
13. Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia–a preliminary report. J Emerg Med. 2000;19:311–5, doi: 10.1016/S0736-4679(00)00255-9.
14. Fagan GJ, Wayment H, Morris DL, Crosby PA. The albumin cobalt binding test: analytical performance of a new automated chemistry assay for the detection of ischemia modified albumin (IMA). J Clin Ligand Assay. 2002;25:178–87.
15. Gidenne S, Ceppa F, Fontan E, Perrier F, Burnat P. Analytical performance of the albumin cobalt binding (ACB) test on the Cobas MIRA Plus analyzer. Clin Chem Lab Med. 2004;42:455–61, doi: 10.1515/CCLM.2004.079.
16. Bennett CM, Guo M, Dharmage SC. HbA(1c) as a screening tool for detection of type 2 diabetes: a systematic review. Diabet Med. 2007;24:333–43, doi: 10.1111/j.1464-5491.2007.02106.x
17. Ukinc K, Eminagaoglu S, Ersoz HO, Erem C, Karahan C, Hacihasanoglu AB, et al. A novel indicator of widespread endothelial damage and ischemia in diabetic patients: ischemia-modified albumin. Endocrine. 2009;36:425–32, doi: 10.1007/s12020-009-9236-5.
18. Bragadeesh T, Sari I, Pascotto M, Micari A, Kaul S, Lindner JR. Detection of peripheral vascular stenosis by assessing skeletal muscle flow reserve. J Am Coll Cardiol. 2005;45:780–5, doi: 10.1016/j.jacc.2004.11.045.
19. Wu WC, Mohler E3rd, Ratcliffe SJ, Wehrli FW, Detre JA, Floyd TF. Skeletal muscle microvascular flow in progressive peripheral artery disease: assessment with continuous arterial spin-labeling perfusion magnetic resonance imaging. J Am Coll Cardiol. 2009;53:2372–7, doi: 10.1016/j.jacc.2009.03.033.
20. Kaefer M, Piva SJ, De Carvalho JA, Da Silva DB, Becker AM, Coelho AC, et al. Association between ischemia modified albumin, inflammation and hyperglycemia in type 2 diabetes mellitus. Clin Biochem. 2010;43:450–4, doi: 10.1016/j.clinbiochem.2009.11.018
21. Dahiya K, Aggarwal K, Seth S, Singh V, Sharma TK. Type 2 diabetes mellitus without vascular complications and ischemia modified albumin. Clin Lab. 2010;56:187–90.
22. Piwowar A, Knapik-Kordecka M, Warwas M. Ischemia-modified albumin level in type 2 diabetes mellitus-Preliminary report. Dis Markers. 2008;24:311–7.
23. Aronow WS, Ahn C, Weiss MB, Babu S. Relation of increased hemoglobin A(1c) levels to severity of peripheral arterial disease in patients with diabetes mellitus. Am J Cardiol. 2007;99:1468–9, doi: 10.1016/j.amjcard.2006.12.085.
24. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. JAMA. 1995;274:1049–57, doi: 10.1001/jama.274.13.1049
25. Darius H, Pittrow D, Haberl R, Trampisch HJ, Schuster A, Lange S, et al. Are elevated homocysteine plasma levels related to peripheral arterial disease? Results from a cross- sectional study of 6880 primary care patients. Eur J Clin Invest. 2003;33:751–7, doi: 10.1046/j.1365-2362.2003.01196.x