Advanced Glycation End Products in Patients with Leukoaraiosis

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Key words

Advanced glycation end products (AGEs) · Diabetes mellitus · Leukoaraiosis

Abstract

Objective: To investigate the relationship between leukoaraiosis and Advanced glycation end products (AGEs). ***Background:*** Accumulation of AGEs in the skin, serum, and other specimens obtained from diabetic patients has been linked to the progression of microvascular complications. Moreover, tissue accumulation of the AGEs can be measured by skin autofluorescence. ***Methods:*** 220 healthy Chinese adults were studied. The screening system included medical and neurological examination, head MRI scans, and blood tests. Leukoaraiosis was diagnosed with T1-weighted, T2-weighted, and FLAIR images of MRI. 49 cases were leukoaraiosis without diabetes mellitus; 52 cases were leukoaraiosis with diabetes mellitus. ***Results:*** Skin AGE levels in patients with leukoaraiosis and diabetes mellitus were significantly increased compared with the control group (2.11±0.12, 2.17±0.176, 2.07±0.129 and 1.918±0.127, respectively, $p<0.01$). However, there was no significant difference in AGEs between the leukoaraiosis and the diabetes mellitus control. ***Conclusions:*** The measurement of skin autofluorescence is a non-invasive and convenient method for AGEs assessment. The result of this study suggested that AGEs can be one of the biomarkers for predicting leukoaraiosis.

Introduction

Cerebral small vessel disease (SVD) can cause stroke and vascular dementia. Small discrete lacunar infarcts and more diffuse areas of chronic ischaemia can result in diseases of the small end vessels of the brain, which can be visualized by MRI. The latter stage is leukoaraiosis, which can increase the risk of stroke, cognitive decline, and dementia [1-3]. Cerebral SVDs can strongly accelerate the manifestation of Alzheimer's disease and other neurodegenerative diseases that would otherwise have occurred much later. As of now, the pathogenesis of cerebral SVDs remains unclear.

Nonenzymatic glycation of proteins is a series of complex and sequential reactions collectively called the Maillard reaction. The early stage reactions lead to the formation of the early glycation adducts, and the later-stage reactions subsequently form Advanced Glycation End products (AGEs). Many cells express surfaces receptors of AGEs, resulting in AGEs-mediated cellular function changes. Receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin family of cell surface molecules whose repertoire of ligands include AGEs, amyloid fibrils, amphoterins and S100/calgranulins. RAGE is identified in neurons, axons, myelin and oligodendrocytes over regions of leukoencephalopathy [4]. RAGE is up-regulated widely within neurons in the brain, particularly in the white matter regions. The heterogenous expression of RAGE over such regions may be a clue to the role of RAGE in the development of leukoencephalopathy [5].

Accumulation of AGE adducts on long-lived proteins reflects the cumulative effects of oxidative stress and hyperglycaemia that are strongly related to the development of diabetic neuropathy. Accumulation of AGEs in the skin, serum, and other specimens obtained from diabetic patients has been linked to the progression of microvascular complications. AGEs also play a role in the pathogenesis of several other diseases, such as renal failure, atherosclerosis and Alzheimer's disease [7, 8, 9].

Skin AGEs levels can be used for evaluating diabetic complications, which also shows to the progression of microvascular complications. Moreover, tissue accumulation of AGEs can be measured by skin autofluorescence [7]. Several studies have shown that skin autofluorescence measurement with the AGE-reader is strongly related to AGE accumulation in healthy subjects, diabetic patients and hemodialysis patients [7, 8]. Because skin autofluorescence reflects tissue

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AGEs accumulation, this technique can be used for the assessment of the risk of AGE-related leukoaraiosis.

We hypothesized that advanced glycation end products, which have previously been implicated in the development of other diabetic complications, might contribute to the pathomechanism for the brain damage during leukoaraiosis. The cross-sectional study described is this paper was designed to test the hypothesis that skin autofluorescence, an indirect measure of skin AGE accumulation, correlates with the development of leukoaraiosis.

Patients and Methods

Subjects

We studied 220 healthy Chinese adults, who voluntarily visited the Anhui Medical University affiliated Anhui Province hospital. The screening system included medical and neurological examination, head MRI scans, and blood tests. Leukoaraiosis was diagnosed with T1-weighted, T2-weighted, and FLAIR images of MRI, all were registered as Grade 9 according to duanping Liao [11]. Forty nine cases were leukoaraiosis without diabetes mellitus; Fifty-two cases were leukoaraiosis with diabetes mellitus. The selection criteria were as follows: informed consent to this study, no history of psychiatric or neurological diseases including transient ischemic attack, no neurological abnormalities. We excluded patients with renal dysfunction, heart disease, and diabetes mellitus with complication. The study was approved by the local research ethics committee,and all patients gave written informed consent.

Table 1 Demographic Data of AGEs and leukoaraiosis

	No LA (n=10)	LA (n=101)
Sex(male)	68	62
Age	58	63
Smoking%	14.65	34.69
Dyslipidemia(TG>150mg/dl)%	11.46	26.53
Elevated Blood Pressure, %	23.57	42.86
Elevated Fasting Glucose > 0, %	14.01	12.24

Magnetic Resonance Imaging

Head MRIs were obtained using conventional pulse sequences for T2-weighted image, T1-weighted image, and fluid-attenuated inversion recovery (FLAIR) image in the transverse plane with a slice thickness of 7 mm by a 3.0-Tesla MRI (Symphony, Siemens).

Skin autofluorescence

AGE detecting system has been shown like Fig 1. When detecting AGE fluorescence spectrum, the apparatus showed (a) can scan exciation light (wavelength in UV 340- 420nm), illuminating skin at a plurality exciation wavelength, detection a single fluorescence wavelength, it can distinguish fluorescence substance of tissue and get the optimum exciation wavelength. The fluorescence signal would be corrected in order to get rid of noise and reduce error. All measurements were performed at room temperature in a semi-dark environment. After control measurements had been made with the lamp off (dark), fluorescence of the skin was measured 4 times (every 5s) at the volar side of the arm for every volunteer, pay attention to perform the measurement at a normal skin site, i.e. one without visible vessels, scars, lichenification, or other skin abnormalities. Repeated fluorescence measurements were taken over in a different day in control subjects and diabetic patients. The differences between repeated measurements were not dependent on the level of fluorescence. Fluorescence was calculated by automated analysis and was observer independent

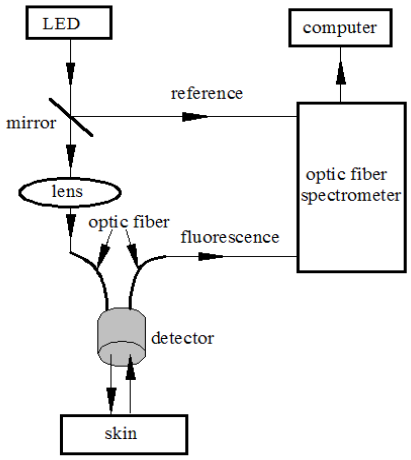

Figure 1 AGE detecting system

Leukoaraiosis Rating

Leukoaraiosis were rated using the Scheltens scale. Each cerebral region was initially scored on the size of the lesions, then on their number. In accordance with this scale, the periventricular white matter hyperintensities were scored on three regions: the frontal and occipital caps, and the periventricular bands. They were rated as none, score 0; 5 mm or less, score 1; and confluent lesions and greater than 5 mm, score 2. The deep white matter hyperintensities were examined in four subcortical regions (frontal, parietal, temporal and occipital lobes). These lesions were rated as none, score 0; 3 mm and smaller and 5 or fewer lesions, score 1; 3 mm or smaller and 6 or more lesions, score 2; 4 to 10 mm and 5 or fewer lesions, score 3; 4 to 10 mm and 6 or more lesions, score 4; 10 mm or larger and 1 or more lesions, score 5; and large confluent lesions, score 6. The total leukoaraiosis score is the sum of the periventricular white matter intensities and deep white matter hyperintensities subscores for a maximum score of 30.

Statistical Analysis

Statistical analysis was done with SPSS 11.0 software (SPSS Inc, Chicago, IL). Univariate associations between potential predictors were analyzed using a t test for continuous variables. Stepwise logistic regression was performed on variables. Results were reported as odds ratio (OR) with associated 95% confidence intervals (CI).

Results

Table 2. The changes of AGE in patients of healthy control, diabetes mellitus control, leukoaraiosis without diabetes, leukoaraiosis with diabetes

Group	**AGE(Mean** ± SD)
Healthy control	1.918±0.127
Diabetes Mellitus Control	2.07±0.129
Leukoaraiosis Without Diabetes	2.11±0.12
Leukoaraiosis with Diabetes	2.17±0.176

Table 2 shows the AGE levels in the 4 groups. Skin AGE levels in patients with leukoaraiosis and diabetes mellitus control were significantly increased compared with the control group (2.11±0.12, 2.17±0.176, 2.07±0.129 and 1.918±0.127,respectively, $p<0.01$). However, there was no significant difference in AGEs between the leukoaraiosis and the diabetes mellitus control.

By univariate logistic analyses, AGEs showed significant associations with leukoaraiosis. Increased age, male sex, and smoking were also significantly associated with leukoaraiosis. Multivariate logistic analyses revealed that increased AGEs, smoking, and elevated fasting glucose were independent risk factors for leukoaraiosis.

Results shows that skin Advanced Glycation End Product Level is significantly associated with leukoaraiosis. The positive trend between skin AGE level and leukoaraiosis could be useful for earlier diagnosing leukoaraiosis. Besides, sex, elevated blood pressure, age and dyslipidemia are also related to the presence of leukoaraiosis. However, smoking seems to have less to do with leukoaraiosis.

Discussion

This is the first report of non-invasive measurement of AGEs in patients with leukoaraiosis. Our study shows that skin autofluorescence is a measure of long-term metabolic burden and seems strongly associated with the presence of leukoaraiosis. Several studies indicate that AGE accumulation in tissue may reflect the cumulative effect of hyperglycemia and oxidative stress over many years.

Accumulation of advanced glycation end products (AGEs) is thought to play a role in the pathogenesis of chronic diabetic vascular complications and renal failure and also to predict long-term complications in age related diseases. In this study, the leukoaraiosis patients with or without diabetes mellitus showed the higher skin autofluorescence compared with control group. Moreover, there is no statistic difference of the skin autofluorescence value between the leukoaraiosis patients with or without diabetes mellitus. This showed the AGEs may play an important in the development of leukoaraiosis.

Although the precise mechanisms underlying leukoaraiosis remain unclear, with Neuroimaging studies, reduced white-matter blood flow, Impaired autoregulation, hypometabolism in affected regions, vascular border zone hypoperfusion and subclinical ischemia were showed for leukoaraiosis patients. Pathological vascular abnormalities reported include both a diffuse arteriopathy of the perforating arteries with hyaline deposition, an appearance referred to as lipohyalinosis, and microatheroma.

Periventricular regions of White Matter Area (WMA) are associated with some evidence of a breakdown of the blood–brain barrier (BBB) and increased blood–brain barrier permeability in well controlled diabetic patients and in association with leukoaraiosis in both patients and controls [13]. Topakian et al declared that leukoaraiosis grade is an independent predictor of these permeability related signal changes, which not only in the areas of white matter lesions (WML) but also in normal appearing white matter (NAWM) [14]. Li et al used streptozotocin-induced diabetic mice to observe the expression of RAGE at the BBB and found Upregulation of RAGE at the BBB and significant AGE-dependent transport of Aβ across the BBB.

Our study shows that the skin AGE values in patients with leukoaraiosis were significantly higher compared with the control group. These results suggested, with AGEs related increased oxidative tress and inflammation, BBB can be broken and become permeable，this may lead to the development of leukoaraiosis. However, Cory et al showed that no significant alteration in blood–brain barrier was detected using immunohistochemistry for immunoglobulin G (IgG) or the vascular cell adhesion molecules ICAM and VCAM. Therefore, evidence of abnormalities in myelination not attributable to vascular ischemia is present throughout many of the areas in which analogous white matter changes occur in the human diabetic brain. Therefore, the accumulation of AGEs in nervous system tissue and the propensity for RAGE upregulation in regions with AGE deposition is a potential pathophysiology of diabetic brain complications. Our study also support the same conclusion.

Yuko Ohnuki et al found Skin AGEs levels in patients with chronic cerebral infarction and silent brain infarction were significantly increased

compared with the control group. In our study, there are 9 metabolic syndrome cases in leukoaraiosis patients without diabetes. Metabolic syndrome (MetS) was significantly associated with every grade of leukoaraiosis. It showed the AGEs may promote the development of leukoaraiosis.

The measuring skin autofluorescence is a non-invasive and convenient method for AGEs assessment. The result of this study suggested that AGEs can be one of the biomarker for predicting leukoaraiosis. The results of this study lead to the following questions: What is the relationship between skin AGEs level and leukoaraiosis MRI Scores; what is the relationships between skin AGEs level and ischaemic stroke subtypes; is the Upregulation of RAGE contributing to the developments of vascular dementia and Alzheimer disease? These questions need further studies to be addressed.

References

1. Vermeer SE, Hollander M, van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MM. et al.. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the Rotterdam Scan Study.*Stroke*.2003;34(5):1126-9
2. Prins ND, van Dijk EJ, den Heijer T, Vermeer SE, Koudstaal PJ, OudkerkM, et al.. Cerebral white matter lesions and the risk of dementia. *Arch Neurol*. 2004;61(10):1531–1534.
3. Au R, Massaro JM, Wolf PA, Young ME, Beiser A, Seshadri S, et al. Association of white matter hyperintensity volume with decreased cognitive functioning: the Framingham Heart Study. *Arch Neurol*. 2006; 63(2):246 –50.
4. Ma, L., Carter, R. J., Morton, A. J. and Nicholson LF.: RAGE is expressed in pyramidal cells of the hippocampus following moderate hypoxic-ischemic brain injury in rats. *Brain Res.* 2003 21;966(2):167-74.
5. Toth C, Schmidt AM, Tuor UI, Francis G, Foniok T, Brussee V.: Diabetes, leukoencephalopathy and rage *Neurobiol Dis.* 2006;23(2):445-61.
6. Liu LP, Hong H, Liao JM, Wang TS, Wu J, Chen SS: Upregulation of RAGE at the blood-brain barrier in streptozotocin-induced diabetic mice Synapse. 2009;63(8):636-42
7. Meerwaldt R, Graaff R, Oomen PH, Links TP, Jager JJ, Alderson NL,et al: Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* 2004, 47(7):1324-1330.
8. Meerwaldt R, Links T, Graaff R, Thorpe SR, Baynes JW, Hartog J, etal.: Simple noninvasive measurement of skin autofluorescence. *Ann N Y Acad Sci* 2005, 1043:290-8.
9. Meerwaldt R, Hartog JW, Graaff R, Huisman RJ, Links TP, den Hollander NC. Skin autofluorescence,a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *J Am Soc Nephrol* 2005.16(12): 3687-93
10. Ohnuki Y, Nagano R, Takizawa S, Takagi S, Miyata T. Advanced Glycation End Products in Patients with cerebral infarction. *Intern Med*. 2009;48(8):587-91.
11. Liao D, Cooper L, Cai J, Toole JF, Bryan NR, Hutchinson RG,, et al. Presence and severity of cerebral white matter lesions and hypertension, its treatment, and its control: The ARIC Study. *Stroke* 1996;27(12):2262–70
12. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults.Executive Summary of The Third Report of The National CholesterolTreatment of High Blood Cholesterol In Adults (Adult Treatment PanelIII). *JAMA*. 2001;16;285(19):2486 –97.
13. Starr JM, Wardlaw J, Ferguson K, MacLullich A, Deary IJ, Marshall I..Increased blood-brain barrier permeability in type II diabetes demonstrated by gadolinium magnetic resonance imaging. *J Neurol Neurosurg Psychiatry*. 2003;74(1):70-6
14. Topakian R, Barrick TR, Howe FA, Markus HS Blood-brain barrier permeability is increased in normal appearing white matter in patients with lacunar stroke and leukoaraiosis. *J Neurol Neurosurg Psychiatry*. 2009.
15. Ruchoux MM, Brulin P, Leteurtre E, Maurage CA.. Skin biopsy value and leukoaraiosis *Ann N Y Acad Sci*. 2000;903:285-92
16. Park K, Yasuda N, Toyonaga S, Yamada SM, Nakabayashi H, Nakasato M Significant association between leukoaraiosis and metabolic syndrome in healthy subjects. *Neurology*. 2007 4;69(10):974-8.