Analysis of risk factors in patients with leukoaraiosis

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Abstract
To investigate the risk factors for leukoaraiosis (LA) and the correlation between risk factors and LA.

The study comprised 92 patients with diagnoses of LA (LA group) and 56 non-LA individuals (control group). Data were collected for the following: age, gender, fasting blood glucose, total cholesterol, triglyceride, high- and low-density lipoprotein cholesterol, uric acid, creatinine, and histories of smoking, hypertension, and diabetes. Levels of serum asymmetric dimethylarginine (ADMA) were detected by enzyme-linked immunosorbent assay.

Univariate analysis showed statistical significance between the 2 groups in age, histories of hypertension and smoking, uric acid, creatinine, and levels of serum ADMA ($P<0.05$). Binary logistic analysis showed that age ($P<0.0001$), hypertension ($P=0.0101$), and serum ADMA ($P=0.0206$) were related to LA. Pearson correlation analysis showed that levels of serum ADMA correlated with uric acid ($r=0.184$, $P=0.025$) and creatinine ($r=0.169$, $P=0.04$).

Age, hypertension, and levels of serum ADMA were independent risk factors for LA. Serum ADMA levels may be related to uric acid and creatinine.

Abbreviations: ADMA = asymmetric dimethylarginine, CT = computed tomography, FBG = fasting blood glucose, HDL-C = high-density lipoprotein cholesterol, LA = leukoaraiosis, LDL-C = low-density lipoprotein cholesterol, MRI = magnetic resonance imaging, NADPH = nicotinamide adenine dinucleotide phosphate, NO = nitric oxide, NOS = nitric oxide synthase, ROS = reactive oxygen species, XOR = xanthine oxidoreductase.

Keywords: age, asymmetric dimethylarginine, hypertension, leukoaraiosis, uric acid

1. Introduction
Leukoaraiosis (LA) is a type of cerebral small-vessel disease diagnosed by computed tomography (CT) and magnetic resonance imaging (MRI). Cerebral small-vessel disease always precedes ischemic or hemorrhagic stroke, and these cerebrovascular accidents are common. LA was first described by Canadian neurologist Hachinski et al.[1] in 1987 as diffuse mottling or senile vascular dementia.[2] Recognition of LA is difficult because of its insidious onset, but the identification of risk factors has become important.[3,4]

It has been shown that changes in brain microvascular endothelial activity can lead to cerebral small-vessel disease, which is considered the major pathogenesis of LA.[5] The dysfunction of vessel endothelial cells can result in increased permeability of the blood–brain barrier. Nitric oxide (NO) is an important vasoactive mediator in endothelial cells. NO is produced by the nitric oxide synthase (NOS) enzyme family, by catalyzing L-arginine (L-Arg). L-Arg has important roles in maintaining the normal function of endothelium.

Asymmetric dimethylarginine (ADMA) is an inhibitor of endogenous NOS, and is produced by endothelial cells. It competes with L-Arg to bind NOS, resulting in reduced NO bioavailability and dysfunction of blood vessel endothelium.[6]

There is little evidence that ADMA is related to LA. In the present study, we analyzed the levels of serum ADMA and several other potential risk factors for LA, to determine the association between LA and risk factors. We hope this prospective study will contribute to the treatment and prevention of LA, and reduce the incidence of stroke through active intervention of risk factors.

2. Methods
2.1. Subjects
A total of 148 subjects were enrolled in the study. Subjects were selected during admission to the Department of Geriatrics and Medical Center at the Second Affiliated Hospital of Harbin Medical University from December 2012 to July 2013. All patients were subjected to examination with a Philips 3.0T cerebral MRI.
A diagnosis of LA was determined if the white matter of the lateral ventricle or centrum semiovale contained diffuse hyperintensities on MRI T2-weighted or fluid-attenuated inversion recovery MRI images, and hypointensities on CT.\textsuperscript{[7–9]} Patients were excluded from this study if they had acute cerebral infarction, cerebral hemorrhage, epilepsy, normal pressure hydrocephalus, intracranial tumors, cranio-cerebral trauma, or radiation encephalopathy; hypothyroidism; severe heart, lung, liver, or kidney dysfunction; central nervous system infection, carbon monoxide poisoning, or immune system disease; or had taken hormone, angiotensin-converting-enzyme inhibitor, statins, or other drugs that could influence levels.

Ninety-two patients (54 men and 38 women; average age 73.97±9.12 years) received a diagnosis of LA, and 56 patients without LA (24 men and 32 women; average age 57.75±8.57 years) comprised the control or non-LA group. The experimental protocol was performed in accordance with the ethical guidelines of the Helsinki Declaration and was approved by the local ethics committee at the Second Affiliated Hospital of Harbin Medical University, China. All patients signed the informed consent form and agreed to participate in the study.

2.2. Clinical data

The clinical data collected from the medical records included age, gender, and histories of smoking, hypertension, and diabetes. Blood samples (5mL) were collected from the ulnar vein in the morning. Fasting blood glucose (FBG), total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), uric acid, and creatinine were detected with an automatic biochemistry analyzer in the clinical laboratory.

2.3. Detection of levels of serum ADMA

Blood serum was collected by centrifuge at 1409g for 20min, and stored at −70°C. Levels of ADMA were detected using an enzyme-linked immunosorbent assay in accordance with the manufacturer’s instructions (RayBiotech, Norcross, GA).

2.4. Statistical analysis

Data with normal distribution were analyzed by 1-tailed t test. Data with non-normal distribution were analyzed using the Mann–Whitney U test. The enumeration data were processed with the chi-squared ($\chi^2$) test. Multiple regression analysis with the forward logistic regression method was used for the analysis of risk factors. In addition, Pearson correlation analysis was used to analyze the correlation between LA and control groups. The specific following risk factors: uric acid, creatinine, age, FBG, total cholesterol, triglyceride, HDL-C, and LDL-C. Statistical analyses were performed with SPSS 16.0 software. $P<0.05$ was considered statistically significant.

3. Results

3.1. Single-factor analysis

The single-factor analysis showed that, of the 13 risk factors considered, age, histories of hypertension and smoking, uric acid, creatinine, and ADMA were statistically significant between the LA and control groups ($P<0.05$; Tables 1–3).

3.2. Binary logistic analysis

For the binary logistic analysis a diagnosis of LA was taken as the dependent variable, and all possible risk factors were independent variables. All clinically relevant variables were included in the model. Linear associations between the independent variable and each dependent variable were analyzed with a scatterplot matrix diagram. The residual plot and dependent variables of the model had a binomial distribution. The results showed that age ($P<0.0001$), hypertension ($P=0.0101$), and levels of serum ADMA ($P=0.0206$) were relatively independent risk factors for LA. The relative risks were 10.492, 1.242, and 5.811, respectively (Table 4).

3.3. Pearson correlation analysis of risk factors and serum ADMA levels

Pearson correlation analysis showed that serum ADMA levels positively correlated with uric acid ($r=0.184$, $P<0.05$) and creatinine ($r=0.169$, $P<0.05$). There was no significant correlation with age, FBG, total cholesterol, triglyceride, HDL-C or LDL-C (Table 5).

4. Discussion

With the aging of populations and the development of imaging techniques, the numbers of patients identified as having LA is...
increasing. It is recognized that ischemic LA is a biological indication of brain aging.[19] Nevertheless, multiple risk factors contribute to LA pathogenesis. LA is associated with dementia, depression, gait disturbance, and falling accidents, as well as lacunar infarction and hemorrhage of the basal ganglia or subcortical structure.[11,12] Thus, LA has a significant negative influence on patients’ quality of life.

We studied 13 potential risk factors for LA, and found that the LA group was significantly and positively associated with age, history of hypertension, smoking, uric acid, creatinine, and ADMA. However, the binary logistic analysis showed that age, hypertension, and levels of serum ADMA are independent risk factors of LA. By excluding confounding factors, the results of the binary logistic analysis were more reliable than the single-factor analysis. The small R score may be related to the sample size, which is not large enough. This is a limitation of the present study.

Age strongly correlates with LA,[13] and our study also demonstrated that age was positively related to LA. Taylor et al.[45] reported that age is an important factor associated with increasing volume of white matter lesions, and studies show that LA prevalence increases with age.[4] This may be because brain proteins such as myelin basic protein and phosphatidylcholine decrease gradually in adulthood. Also, most nerve fibers in white matter are myelinated nerve fibers; the length of myelinated axons is greatly reduced in normal aging process. In addition, atherosclerotic vascular changes increase with age. This decreases the blood supply to white matter and chronic ischemia is possible.

In the present study, we found that hypertension is an independent risk factor for LA. The periventricular white matter is located deep within the watershed area, which receives the blood supply from the long circulation branches of the meningeal and ventricular arteries of the subependymal artery. Thus, less collateral circulation exists. Long-term chronic hypertension may cause wall thickening, hyaline degeneration, and narrowing in the small intracranial artery and branches. This leads to circulatory disturbance, and secondary hypoxic ischemic demyelination occurs in the deep white matter.[11] Hypertension can induce angioedema and damage of the blood–brain barrier, leading to greater permeability and the leakage of cerebrospinal fluid protein and LA.[16] Severe acute hypertension can cause several pathological changes, including the increase of plasma osmotic pressure of the capillary arterioles, regional brain edema, glial cell proliferation, and demyelination. It has been reported that periventricular white matter lesion is linked to both diastolic pressure and systolic pressure.[17] Vuorinen et al.[18] considered that hypertension would increase the risk of LA from middle to old age. Their study suggests that early control of hypertension could reduce the incidence of LA with age.

ADMA acts as an endogenous inhibitor of NOS and leads to endothelial dysfunction because of the inhibition of NO synthesis. It has been proposed as a new predictor of endothelial dysfunction.[19] Vascular endothelial cells synthesize and secrete various bioactive substances to maintain vasoconstriction and vasodilatation, vascular tone,[20] normal blood flow, and long-term vascular patency.[21] However, endothelial cells in cerebral vessels participate in the impairment of the blood–brain barrier,[22] and are essential for the function of cerebral blood flow autoregulation.[18] It is commonly accepted that impairment of the blood–brain barrier, cerebrovascular autoregulation, and chronic hypoperfusion are the important mechanisms for LA development. One study has shown that in elderly cardiovascular patients, endothelium-dependent vascular relaxation negatively correlates with the volume of hyperintense regions in white matter,[23] which is not associated with endothelium-independent vasorelaxation. Therefore, vascular endothelial dysfunction potentially underlies the development of white matter lesions. It also indicates that ADMA may disrupt the blood–brain barrier and take part in forming white matter lesions by causing vessel endothelial dysfunction.

Elevated ADMA levels can damage endothelium-dependent vasodilatation,[24] and affect cognitive functioning in patients
with LA.\textsuperscript{[25]} Many risk factors such as hypertension, hypercholesterolemia, smoking, diabetes mellitus, and vascular inflammation may contribute to vascular disease through the NO pathway.\textsuperscript{[26]} In a study of the effect of NOS inhibition on dynamic cerebral autoregulation, White et al.\textsuperscript{[27]} confirmed that NO is involved in cerebral hemodynamic autoregulation. ADMA may act as a nonspecific inhibitor of NOS to inhibit NO formation to destroy cerebral hemodynamic autoregulation. Researchers injected ADMA into the veins of healthy volunteers in a randomized, double-blind, placebo-controlled clinical study.\textsuperscript{[28]} The cerebral blood flow in the ADMA group was less than that of the placebo group. Thus, ADMA could significantly reduce cerebral perfusion and vascular compliance. Many studies have concerned the association between elevated ADMA and stroke.\textsuperscript{[29,30]} However, there are few that have investigated a link between ADMA and cerebral small-vessel disease.\textsuperscript{[31,32]} Only a few studies report an association between serum ADMA and white matter lesions. Khan et al.\textsuperscript{[33]} reported that the average serum ADMA concentration in patients with cerebral small vessel disease was higher than that of the controls; the extent of cerebral white matter lesions correlated with serum ADMA levels. Nonetheless, there was no correlation between lacunar infarction and ADMA. Pikula et al.\textsuperscript{[34]} discovered that high ADMA levels could be linked to the incidence of silent cerebral infarction, which is associated with cerebral white matter lesion. In the present study, we found through single-factor analysis and binary logistic analysis that high serum ADMA is an independent risk factor for LA. That is, the higher the concentration of serum ADMA, the greater the risk of LA. Thus, ADMA may be a newly reported risk factor for LA. ADMA may be involved in the development of LA by causing endothelial dysfunction, cerebrovascular autoregulation disorder, a decrease in cerebral perfusion, and damage to the blood–brain barrier. In addition, the present study showed that serum ADMA levels positively correlated with uric acid and creatinine. Uric acid is an end product from purine derivatives in human metabolism, and the level of serum uric acid is affected by various factors. Excessive production or reduced excretion of uric acid leads to hyperuricemia. Recent studies confirm that uric acid can convert from an antioxidant into a pro-oxidant under certain conditions, to impair endothelial function.\textsuperscript{[35]} Increased uric acid levels are associated with hypertension, coronary heart disease, acute stroke, and other cardiovascular diseases.\textsuperscript{[36,37]} It is not known whether uric acid is protective or a risk factor with regard to white matter lesions. In the present study, the univariate analysis showed that the uric acid level of the LA group was significantly higher than that of the non-LA group. However, according to the binary logistic analysis, the difference was not significant and uric acid was not relevant in LA. Pearson correlation analysis showed that serum ADMA levels positively correlated with uric acid, meaning that uric acid may increase the risk of LA by influencing the level of ADMA. The effect of uric acid on serum ADMA levels is likely related to oxidative stress in humans. Reactive oxygen species (ROS) is a major source of oxidative stress.\textsuperscript{[38]} Xanthine oxidoreductase (XOR) and nicotinamide adenine dinucleotide phosphate oxidase (NAPDH oxidases) are the key enzymes of vascular endothelial cells for producing ROS. XOR catalyzes the conversion of xanthine to uric acid and produces the oxidant O$_2^-$—during purine metabolism. XOR levels are low in normal physiological conditions. However, XOR levels increase when inflammatory injury occurs, thus the production of uric acid and ROS rise.\textsuperscript{[39]} In addition, uric acid promotes ROS production by stimulating NAPDH oxidases in endothelial cells.\textsuperscript{[40]} In general, the human body produces 300 $\mu$mol of ADMA daily, with about 50 $\mu$mol ADMA excreted by the kidneys.\textsuperscript{[41]} Our data in the present study showed that serum ADMA levels positively correlated with creatinine concentration. Although the association between creatinine and uric acid remains to be examined, the increase of creatinine with renal hypofunction is often accompanied by an increase of uric acid. Therefore, an increase in ADMA with uric acid may be due to renal hypofunction. In conclusion, old age, hypertension, and elevated serum ADMA levels are high-risk factors for LA. Renal hypofunction and high serum uric acid may increase the level of serum ADMA. Age is an irreversible factor, but ADMA levels may be lowered by controlling hypertension, protecting renal function, and decreasing uric acid, which in turn may reduce the likelihood of LA. Serum ADMA levels may indicate the presence of LA.

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