ORIGINAL RESEARCH

Investigation of ischemic and demyelinating lesions by cerebral vasoreactivity based on transcranial Doppler sonography: a comparative study

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Purpose: Variations of cerebral blood flow in response to hypoxia and hyperoxia in different disease conditions can provide new insights into disease etiopathogenesis. This study aimed to determine the characteristics of cerebral vasoreactivity for ischemia and demyelination.

Materials and methods: This case–control study included: 28 patients with lacunar infarctions verified by history, physical examination, and MRI; 28 age- and sex-matched healthy controls; 28 patients with relapsing–remitting multiple sclerosis (MS), based on McDonald criteria; and 28 age- and sex-matched healthy controls for the MS group. Transcranial Doppler sonography was undertaken in all subjects to calculate the mean flow velocity (MFV) of the right middle cerebral artery (MCA) and, after a breath-holding (BH) maneuver, the breath-holding index (BHI) was determined.

Results: There was no significant difference of BHI and changes of MFV of the MCA in MS patients compared to controls (1.02 ± 0.4 vs 1.02 ± 0.3, $p = 0.993$; and 16.8 ± 8.1 vs 11.3 ± 10.8, $p = 0.057$). BHI in patients with lacunar infarctions was significantly lower (0.8 ± 0.4 vs 1.2 ± 0.3, $p < 0.001$) compared to controls. The BHI ($p = 0.040$) and variations of MFV of MCA ($p = 0.007$) in MS patients were significantly higher than in patients with lacunar infarctions. The vasoreactivity of demyelinating lesions was higher than that of ischemic ones.

Conclusion: Therefore, cerebral vasoreactivity determined by transcranial Doppler could be utilized for differentiating demyelinating from ischemic lesions.

Keywords: cerebral vasoreactivity, multiple sclerosis, ischemia, demyelination

Introduction

Stroke is among the leading causes of morbidity, mortality, and disability among both developed and developing countries, accounting for approximately 15 million new cases and five million deaths annually.[1,2] Approximately 85% of all strokes are ischemic and 10%–15% are reported to be hemorrhagic.[3] Lacunar infarction is the result of ischemia in small penetrating arteries called lacunes, and it has unique clinical and pathological features. It has been demonstrated that cerebral autoregulation is impaired after ischemia and stroke due to the ischemic damage to cerebral arterioles and capillaries.[4] Other chronic conditions affecting blood vessels such as hypertension can also result in impaired cerebral autoregulation.[5] In other words, ischemia results in endothelial cell and receptor dysfunction as well as smooth muscle activation, leading to impaired cerebral autoregulation.[6]

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating, and degenerative disease of the central nervous system with an estimated prevalence of 2.93 per 100,000 inhabitants that has a subtle and undetermined pathological mechanism. An acute MS lesion is characterized by hyperintense lesions in T2-weighted and

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diffusion-weighted (DW) brain MRI and hypointense lesions on apparent diffusion coefficient (ADC) imaging that are similar to findings in acute lacunar ischemia. Thus, it is important to differentiate these lesions from ischemic changes.[7] Proposed mechanisms of MS include inflammatory changes, demyelination, axonal degeneration, and oligodendrocyte loss.[8] However, recent studies have revealed a close relationship between cerebral vasculature, blood flow, and MS.[9,10] Histopathological studies of MS patients have revealed several microvascular changes in cerebral tissue including thickening or hyalinization of vein walls and small veins and capillary thrombosis suggesting an ischemic basis for the disease.[11] As mentioned above, lacunar infarction and MS plaques share similar characteristics in T2-weighted and DW brain MRI and ADC imaging. This intensifies the differentiation between these two conditions and further management, because management of these two condition is entirely different. However, it is suggested that cerebrovascular reactivity is normal in different disease activity levels of MS.[10] Thus, evaluating cerebral vasoreactivity may be an appropriate tool for differentiating ischemic lesions from demyelinating ones.

Cerebral vasoreactivity could be evaluated through different methods including the transcranial Doppler (TCD) sonography,[12] dynamic susceptibility contrast perfusion MR imaging,[13] hypercapnia MRI study,[9] and measurement of circulating endothelin-1 and asymmetric dimethylarginine.[14] Flow velocities in the large cerebral arteries and velocity changes after vasodilatory stimulus, such as acetazolamide, CO_2, or apnea, could be evaluated by means of TCD sonography.[15] Increased CO_2 levels induced by breath holding results in an autoregulatory vasodilatation, and an increase in cerebral blood flow (CBF) to the cortex.[16,17] This increase in CBF can provide precious and valuable information about vascular integrity and proper function.[12] As data on the differentiating properties of cerebral vasoreactivity evaluation by means of TCD sonography are scarce, we undertook this study to investigate changes in cerebrovascular vasomotor reactivity induced by breath holding using TCD sonography in patients with MS and lacunar infarction.

Materials and methods
Patients
This case–control study was conducted, during a 12-month period from March 2011 to March 2012 in the Department of Neurology, Nemazee Hospital, and the Motahari Clinic, both tertiary health care centers affiliated with the Shiraz University of Medical Sciences. In this study, we included four groups of participants. The first study group included 28 patients admitted to the Department of Neurology of Nemazee Hospital with a diagnosis of lacunar infarction. Lacunar infarction was diagnosed according to hyperintense lesions on T2-weighted brain MRI. In addition, all patients had DWI sequences, and we included both acute and old lacunar infarctions. All subjects suffered from motor and/or sensory deficit and ataxic signs without signs of cortical involvement. All of the ischemic lesions in these patients were found in the deep brain within the territory of a single perforating artery with a maximum diameter of 15 mm on imaging. A group of 28 healthy volunteers matched by sex and age were included as the control group of subjects with lacunar infarction. Furthermore, we included 28 patients with clinically diagnosed relapsing–remitting (RR) MS according to the McDonald criteria who were referred due to an acute relapse.[15] Hemispheric involvement was confirmed in all of the patients by detecting hyperintense lesions on T2-weighted brain MRI. We also included 28 age- and sex-matched healthy volunteers as controls for the MS group. Healthy volunteers were selected from those referred to the neurology departments of our centers as patients' relatives or at the Motahari clinic for routine follow-ups.

Exclusion criteria for two control groups and patients with MS included any history of transient ischemic attack (TIA) or cerebrovascular accident (CVA), severe carotid artery stenosis (>70%), cardiac problem (atrial fibrillation, recent myocardial infarction [MI], endocarditis, dilated cardiomyopathy), COPD, hypertension (systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg), diabetes mellitus (DM; fasting blood sugar [FBS] >126 mg/dL), hypercholesterolemia (total cholesterol >200 mg/dL), obesity, smoking (>1 pack-year), congestive heart failure (greater than New York Heart Association Grade I), hematologic disease, and cancer. Moreover, we excluded those participants who were being treated with hormonal substances, nitrates, β-blocking agents, calcium channel blockers, anticoagulants, and vasodilatory drugs. Exclusion criteria for those diagnosed with lacunar infarction were severe carotid artery stenosis (>70%), cardiac problem (atrial fibrillation, recent MI, endocarditis, dilated cardiomyopathy), COPD, being treated with hormonal substances, nitrates, β-blocking agents, calcium channel blockers, anticoagulants, and vasodilatory drugs for up to 1 week prior to the study. Case and control groups were matched for sex and age, because these two variables have been proved to affect cerebrovascular vasomotor reactivity as well as the breath-holding index (BHI).[18] Patients with

MS who had acute lacunar infarctions diagnosed with DWI were excluded.

The study protocol was approved by the institutional review board of Shiraz University of Medical Sciences, and the approval of the Ethics Committee was obtained before beginning the study. All participants provided written informed consent before inclusion in the study.

Study protocol

All subjects underwent a careful cardiologic and neurological examination by the neurologist resident and a cardiologist. Electrocardiography (ECG) was conducted in order to rule out the cardiovascular diseases. Laboratory evaluation included FBS, total cholesterol and complete blood count in order to detect those with DM, hypercholesterolemia, and anemia. All subjects underwent Doppler sonography of bilateral carotid arteries in order to rule out severe carotid artery stenosis at the same time as the TCD sonography study.

TCD sonography was carried out in all patients using a TCD DWL Multidop machine (scimed Ltd/TC22) with a 2-MHz, hand-held, pulsed-wave Doppler probe. All the TCDs were undertaken by an experienced neurology resident under the supervision of the neurology attending. TCD sonography was done via a temporal window in a quiet room after at least 5 minutes of rest in the supine position. The probe was adjusted until a maximal ultrasonic signal was obtained. This point was approximately at a depth of 50 mm. The probe was held in position in order to evaluate the TCD sonography parameters.

Mean flow velocity (MFV) in the middle cerebral artery (MCA), was recorded for 1 minute. Thereafter, cerebro-vascular vasoreactivity to hypercapnia was evaluated by the means of a breath-holding maneuver according to the procedure previously described by Markus and Harrison.[12] After normal breathing, the subjects were instructed to hold their breath for 30 seconds. During the breath-holding period, the MFV in the MCA was recorded continuously. Subjects were requested not to perform a Valsalva maneuver during breath holding because it would result in blood pressure (BP) changes and disturb cerebral hemodynamics.

Then, the BHI was calculated by the following formula:

$$\text{BHI}\,(\%) = \frac{\text{MFV(apnea)} - \text{MFV(rest)}}{\text{MFV(rest)} \times 30} \times 100.$$

Statistical analysis

Based on 95% CI and 80% power and to detect significant differences regarding BHI between the four study groups ($p = 0.05$, two-sided), 28 patients were required in each group. To compensate for possible unevaluable data, we enrolled 112 patients in each study group. The Statistical Package for Social Sciences (SPSS) for Windows, version 18.0 (SPSS, Chicago, IL, USA) was used for data analysis. Between-group differences were compared using one-way analysis of variance (ANOVA). For comparison between groups, Bonferroni post hoc test was used. The Mann–Whitney U-test was used for comparison between groups in non-normal distribution. Categorical variables were analyzed using the chi-square test. In order to compensate for confounders, we also ran a multivariate logistic regression model encompassing age and sex as independent factors. Data are reported as mean ± SD and proportions as appropriate. A two-sided p-value of less than 0.05 was considered statistically significant.

Results

A total of 112 subjects in four study groups of lacunar infarction and control as well as MS and control were included in this study. There were 48 (42.9%) men and 64 (57.1%) women among the participants. The mean age of all the participants was found to be 41.1 ± 13.7 (ranging from 19 to 70) years. The baseline characteristics of the patients and the controls are summarized in Table 1.

The mean BHI in those with lacunar infarction was found to be significantly lower when compared to their age- and sex-matched controls (0.8 ± 0.4 vs 1.2 ± 0.3; $p < 0.001$). There was no significant difference between those with lacunar infarction and their controls with regard to MFV_{rest} ($p = 0.264$), $\text{MFV}_{\text{apnea}}$ ($p = 0.227$), and MFV variations ($p = 0.649$) (Table 2). The mean BHI in those with MS was found to be comparable with their age- and sex-matched controls (1.02 ± 0.4 vs 1.02 ± 0.3; $p = 0.993$). There was no significant difference between those with MS and their

Table 1 Baseline characteristics of 112 patients and controls included in the current study

	MS (n = 28)	Control (n = 28)	Lacunar infarction (n = 28)	Control (n = 28)
Age (years)	29.85 ± 6.8	30.57 ± 6.6	52.1 ± 10.2	51.8 ± 9.7
Gender				
Men (%)	7 (25.0%)	7 (25.0%)	17 (60.7%)	17 (35.4%)
Women (%)	21 (75.0%)	21 (75.0%)	11 (39.3%)	11 (39.3%)
Disease duration (months)	36.3 ± 12.6	N/A	N/A	N/A
EDSS	2.83 ± 1.56	N/A	N/A	N/A

Abbreviations: EDSS, Extended Disability Status Scale; ms, multiple sclerosis.

Table 2 TCD sonography characteristics of subjects with lacunar infarction and healthy individuals

	Lacunar infarction (n = 28)	Controls (n = 28)	p-value
BHI (%)	0.8 ± 0.4	1.2 ± 0.3	<0.001
MFV$_{rest}$	47.2 ± 14.2	51.4 ± 13.4	0.264
MFV$_{apnea}$	58.5 ± 17.7	63.9 ± 14.8	0.277
MFV variations	11.2 ± 6.4	12.4 ± 11.9	0.649

Abbreviations: BHI, breath-holding index; MFV, mean flow velocity; TCD, transcranial Doppler.

Table 4 TCD sonography characteristics of subjects with MS and lacunar infarctions

	Lacunar infarction (n = 28)	MS (n = 28)	p-value
BHI (%)	0.8 ± 0.4	1.02 ± 0.4	0.040
MFV$_{rest}$	47.2 ± 14.2	54.8 ± 2.3	0.010
MFV$_{apnea}$	58.5 ± 17.7	74.4 ± 18.6	0.002
MFV variations	11.2 ± 6.4	16.7 ± 8.1	0.007

Abbreviations: BHI, breath-holding index; MFV, mean flow velocity; MS, multiple sclerosis; TCD, transcranial Doppler.

controls with regard to MFV$_{rest}$ ($p = 0.849$), MFV$_{apnea}$ ($p = 0.448$), and MFV variations ($p = 0.057$) (Table 3).

The mean BHI in those with MS was found to be significantly higher when compared to those with lacunar infarction (1.02 ± 0.4 vs 0.8 ± 0.4; $p = 0.040$). In the same way, patients with MS had significantly higher MFV$_{rest}$ ($p = 0.010$), MFV$_{apnea}$ ($p = 0.002$), and MFV variations ($p = 0.007$) when compared to those with lacunar infarctions (Table 4). In order to compensate for confounders such as age and sex, we ran a multivariate logistic regression model. We found that, after compensating for confounders, patients with MS had comparable BHI to those with lacunar infarctions. In addition, the BHI was comparable between the MS patients and the healthy controls of the lacunar infarction group (1.02 ± 0.4 vs 1.2 ± 0.3; $p = 0.863$).

Discussion

In this case–control study, we tried to investigate the differentiating properties of BHI measured by TCD sonography in ischemic and demyelinating brain lesions. We found that BHI was significantly lower in those with lacunar infarcts when compared to their age- and sex-matched healthy controls demonstrating impaired cerebral vasoreactivity in ischemia even in small quantity (lacunar infarction). However, patients with MS were found to have intact cerebral vasoreactivity based on the observation that the BHI was identical between MS patients and healthy controls. Correspondingly, the BHI was significantly higher in MS patients when compared to

Table 3 TCD sonography characteristics of subjects with MS and healthy individuals

	MS (n = 28)	Controls (n = 28)	p-value
BHI (%)	1.02 ± 0.4	1.02 ± 0.3	0.993
MFV$_{rest}$	57.6 ± 14.7	58.6 ± 19.2	0.849
MFV$_{apnea}$	74.4 ± 18.6	69.9 ± 20.7	0.448
MFV variations	16.7 ± 8.1	11.3 ± 10.8	0.057

Abbreviations: BHI, breath-holding index; MFV, mean flow velocity; MS, multiple sclerosis; TCD, transcranial Doppler.

patients with lacunar infarction. This finding demonstrates that BHI might be a reliable and accurate index for differentiating ischemic lesions from demyelinating ones.

Cerebral autoregulation is the innate potential of the cerebral blood vessels to maintain and regulate the CBF in a constant range despite a wide range of systemic BP levels. Cerebral blood vessels undertake autoregulation through a complex of myogenic, neurogenic, and metabolic mechanisms. Several factors are responsible for cerebral autoregulation, the most important of which include vascular conductance and arterial BP.[19] In addition, it has been shown that arterial CO_2 concentrations are the most important factor by which the CBF is regulated.[20] When the cerebral perfusion pressure is modified, blood vessels adapt their vascular resistance in order to maintain the CFB at its baseline.[21,22] The lower and upper limits of cerebral autoregulation in the normotensive human are considered to be at mean arterial pressures of 60 and 150 mmHg, between which the CBF remains relatively constant. Cerebral autoregulation has been found to be damaged in several conditions including head injury,[12] ischemic stroke,[5] and subarachnoid hemorrhage.[23,24] This vicious cycle imposes fluctuations in perfusion pressure in the brain and is associated with worse prognosis.

It has been previously demonstrated that cerebral autoregulation is impaired in ischemic stroke (even in minor stroke), probably secondary to cerebral vasculature endothelial dysfunction as well as venous thrombosis.[5,19] Maintenance of cerebral perfusion during ischemia and stroke is important to conserve and protect the penumbral tissue. Autoregulation is responsible for maintaining cerebral perfusion during stroke and even during interventions in stroke units including BP manipulation, mobilization, and angiographic interventions. However, cerebral autoregulation is impaired secondary to ischemia, and this results in a vicious cycle leading to increased ischemic area and decreased penumbra. It has been demonstrated that cerebral autoregulation is impaired in both hemispheres when an ischemia in the territory of large intracranial vessels occurs. The most interesting finding is

that this bilateral effect seems to be more pronounced in lacunar stroke.[5] It has been demonstrated that the impairment of the cerebral vasoreactivity occurs within the first days after the ischemia, but the recovery lasts for several months.[25,26] Impaired cerebral autoregulation in stroke is related to acute neurological deterioration, necessity of decompressive surgery, and poor outcome. All of these evidences are in concordance with our study that revealed impaired cerebral autoregulation in lacunar infarction.

We found that cerebral autoregulation remains intact in patients suffering from MS when compared to healthy age- and sex-matched controls, and this is in concordance with a similar previous study.[10] Previous studies have determined the cardiovascular autonomic response in MS patients in comparison to healthy individuals. However, they have revealed conflicting results, probably because of different patient selection criteria. Some of these studies have shown that >50% of patients with MS suffer from cardiovascular autonomic dysfunction, whereas some others have reported no significant differences in these patients, similar to our observations in the present study.[27,28] Most studies have reported a significant association between increased MS severity and cardiovascular autonomic dysfunction.[29] In another study, Ozkan et al[30] revealed that blood flow velocities were significantly lower than that in controls during attack periods, and increased during the attack-free period along with clinical improvement. Additionally, vasoreactivity was significantly higher during the attack period, and decreased during the attack-free period; however, it was still significantly higher than in the controls. These findings are in agreement with our study. It is suggested that MS patients have a more reactive posterior circulation than healthy subjects.[10] In this regard, Sloper et al[31] showed that recurrent hypoxia leads to degeneration in GABA terminals of inhibitory neurons as well as a failure in the cortical inhibitory system.

There are several factors affecting cerebral vasoreactivity and brain autoregulation. These factors include aging, vascular sclerosis, DM, and HIV cerebritis.[32–34] Age-related decreases in baseline CBF and CVR are observed in the cerebral cortex, which may be related to the vulnerability for neurological disorders with aging.[32] In our study as the patients with MS had a significantly lower age compared to those with lacunar infarction, it might have affected the results. However, we found that patients with MS had comparable indices with older healthy controls. Thus, we can conclude that lacunar infarctions are associated with decreased cerebral vasoreactivity, which could be exacerbated with advanced age. The other factor that might affect the vasoreactivity

measurement in patients with MS is the volume and number of the lesions.[35] As we have not undertaken MS lesion volumetry, we cannot comment on the issue.

We had some limitations with regard to this study, of which the most important is that it had a small study population. Future studies with larger study populations are needed to shed light on this issue. The other limitation was that patients with lacunar infarction had a significantly higher age when compared to those with MS. In addition, they had a significantly higher male-to-female ratio when compared to the MS group. It is well established that BHI is affected by both age and sex, being lower in women and decreasing by the age especially in women.[18] Although this could be responsible for the lower BHI in MS patients compared to those in the lacunar infarction group, the evidence suggests that impaired cerebral autoregulation is the main reason for this findings.

In conclusion, demyelinating lesions are associated with higher vasoreactivity compared to ischemic ones. In other words, cerebral autoregulation is impaired in ischemic stroke whereas it seems to remain intact in MS. Thus, cerebral vasoreactivity measured by TCD sonography can be used for differentiating ischemic and demyelinating lesions.

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Disclosure
The authors report no conflicts of interest in this work.

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