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The role of homocysteine as a significant risk factor for white matter lesions in Japanese women with rheumatoid arthritis

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Abstract

The presence of white matter lesions (WML) is an important prognostic factor for the development of stroke. Plasma total homocysteine (tHcy), which increases with diabetes, has been flagged as a novel predictor for cerebrovascular events. We tested the hypothesis that the presence of WML correlates with tHcy in rheumatoid arthritis patients. Based on brain magnetic resonance imaging findings, 65 rheumatoid arthritis patients were divided into 2 groups: WML-positive group (61 ± 6 years, mean ± SD; n = 25) and WML-negative group (60 ± 7 years, n = 40). The level of metabolic parameters was assessed by total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting plasma glucose, and homocysteine (tHcy). The duration of rheumatoid arthritis was longer in the WML-positive group than in the WML-negative group ($P < .05$). Plasma levels of triglyceride was higher whereas high-density lipoprotein cholesterol was lower in the WML-positive group than in the WML-negative group ($P < .05$ and $P < .01$, respectively). Fasting plasma glucose ($P < .05$) and tHcy (<.0001) levels were higher in the WML-positive group than in the WML-negative group. Multivariate logistic analysis revealed that WML was independently predicted by the tHcy (odds ratio, 1.35; 95% confidence interval, 1.12-1.63; $P < .0001$). Our findings indicate that the presence of WML was associated with the tHcy in Japanese patients with rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is associated with increased mortality and comorbidity, mostly owing to an excess of cardio- and cerebrovascular disease [1-3].

Since the introduction in the 1980s of brain magnetic resonance imaging (MRI) with its high sensitivity and resolution capacity, changes in the cerebral white matter lesions (WML) are commonly detected in elderly people [4,5]. These lesions have been hypothesized to be ischemic complications of cerebral microvascular disease [6] based on histopathologic studies that demonstrate small-vessel changes in brains with WML and on clinical studies that show associations between WML and microvascular risk factors, such as hypertension [7] and diabetes [8]. In addition, WML is an important prognostic factor for the development of stroke [9,10].

Vascular risk factors, such as hypertension [11] and diabetes [12], are associated with greater lesion burden; and there has been increasing interest in identifying potentially modifiable risk factors. Elevated total plasma homocysteine (tHcy) is currently recognized as a risk factor for cardiovascular and cerebrovascular diseases [13,14]. Homocysteine is a sulfur-containing amino acid. To date, several studies describe the association between the presence of WML and the level of tHcy [14-17].

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The significance of increased tHcy and the presence of WML in RA patients has not been adequately investigated. In this study, we hypothesized that the presence of WML is associated with tHcy in RA patients. To test our hypothesis, we compared the MRI, metabolic profiles, and level of tHcy in Japanese RA patients WML positive and those WML negative, followed by evaluation of the independent predictors of WML in these patients.

2. Materials

2.1. Study population

Women with RA were recruited for a cross-sectional evaluation of RA disease features, cerebrovascular risk factors (ie, homocysteine, metabolic factors), and brain MRI of WML (June 2007 to January 2008). All RA participants were diagnosed after 16 years according to the 1987 revised American College of Rheumatology criteria [18], with disease duration of at least 2 years, and were recruited from the Oita Red Cross Hospital. The first 85 women with RA fulfilling entry criteria and providing written informed consent were enrolled. Twelve patients did not complete the brain MRI, and 2 patients were excluded because of diagnosis misclassification. Of the remaining 71 patients, 6 patients with a prior cardiovascular event (myocardial infarction, angina, or stroke) were excluded from this analysis.

All subjects gave their written informed consent to participate in the study; and the study protocol was approved by the ethics committee of the Oita Red Cross Hospital.

2.2. Traditional cardiovascular risk factors

The study visit included 2 consecutive blood pressure readings (with patients seated) and a fasting blood draw. Total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low density lipoprotein-cholesterol (LDL-C) were measured at the lipid laboratory, Oita Red Cross Hospital.

The LDL-C concentrations in serum were determined by the Friedewald formula [19] from concentrations of total cholesterol, triglycerides, and HDL-C.

The plasma glucose levels were determined by enzymatic assay. *Hypertension* was defined as a previous physician diagnosis of hypertension, measured mean blood pressure of at least 140/90 mm Hg, or use of antihypertensive medication [20].

Dyslipidemia was defined as fasting triglycerides levels of at least 200 mg/dL or an HDL-C concentration less than 45 mg/dL for women and less than 35 mg/dL for men [21]. *Diabetes mellitus* was defined as use of hypoglycemic agent or measured fasting blood glucose greater than 126 mg/dL. Serum total homocysteine levels were determined using the homocysteine microplate enzyme immunoassay (Bio-Rad Laboratories, Oslo, Norway) [22].

2.3. Evaluation of WML

All participating patients underwent brain MRI using a superconducting magnet at a field of 1.5 T on proton density–, T_1-, and T_2-weighted images in axial planes at 5-mm–thick slices. Details of the image interpretation protocols used were the same as those of the Rotterdam study [23]. Patients were classified into the following 2 states: (1) normal scans (negative), if there was either absent or only slight periventricular hyperintensity (small caps or pencil-thin lining), fewer than 5 focal lesions, and no confluent lesions; or (2) abnormal scans (positive), if there was moderate or severe periventricular hyperintensity, 5 or more focal lesions, or confluent lesions. All MRI scans were examined by 2 raters who were blinded to all clinical information. In case of a disagreement of more than 1 point, a consensus reading was held; in all other cases, the readings of both readers were averaged. The interrater and intrarater studies showed a good to excellent agreement.

2.4. Statistical analysis

All data were summarized as the means ± SD. Differences between the groups were examined for continuous variables using the Student *t* test and for categorical variables with the χ^2 test. Logistic regression analysis was used to assess the influence of explanatory variables on WML, where the explanatory variables were hypertension, dyslipidemia, diabetes, blood pressure, heart rate, total cholesterol,

Table 1
Clinical characteristics of studied patients (N = 65)

	WML negative	WML positive	*P* value
Age (y)	60 ± 7	61 ± 6	NS
n	40	25	NS
Body mass index (kg/m^2)	23.2 ± 2.1	23.6 ± 2.6	NS
Duration of RA (y)	7.1 ± 5.4	10.6 ± 7.4	.0316
Hypertension (%)	38	40	NS
Dyslipidemia (%)	33	36	NS
Diabetes mellitus (%)	23	28	NS
Drug use (%)			
NSAID	72	76	NS
DMARD	56	60	NS
Methotrexate	51	52	NS
Steroid	85	88	NS
Systolic blood pressure (mm Hg)	130 ± 11	133 ± 8	NS
Diastolic blood pressure (mm Hg)	75 ± 7	76 ± 9	NS
Heart rate (beats/min)	67 ± 7	68 ± 8	NS
Total cholesterol (mg/dL)	190 ± 34	202 ± 39	NS
Triglyceride (mg/dL)	128 ± 30	146 ± 36	.0309
HDL-C (mg/dL)	47 ± 7	42 ± 8	.0086
LDL-C (mg/dL)	118 ± 35	131 ± 30	NS
FPG (mg/dL)	111 ± 16	119 ± 13	.0393
Creatinine (mg/dL)	1.0 ± 0.2	1.1 ± 0.2	NS
CRP (mg/dL)	1.09 ± 1.46	1.16 ± 1.33	NS
ESR (mm/h)	19.6 ± 6.3	22.9 ± 9.3	NS
Homocysteine (μmol/L)	11.8 ± 4.2	21.0 ± 6.9	<.0001

Data are means ± SD. NSAID indicates nonsteroidal anti-inflammatory drug; DMARD, disease-modifying antirheumatic drug; NS, not significant.

triglyceride, HDL-C, fasting plasma glucose (FPG), creatinine, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and tHcy, and where hypertension, dyslipidemia, and diabetes were dichotomized as 1 (presence) and 0 (absence) by cutoff values defined in the previous section. For WML, the positive was represented as 1 and negative as 0. To determine the best model, that is, for selecting significant factors among the all explanatory variables used, a backward elimination procedure was used. According to the procedure, the duration of RA, triglyceride, HDL-C, FPG, and tHcy were determined as significant factors influencing WML.

Differences were considered statistically significant at P less than .05. All calculations were performed using a standard statistical package (JMP 6.0; SAS Institute, Cary, NC).

3. Results

Women with RA were classified into 2 groups on the basis of the presence or absence of WML on the brain MRI scans. Twenty-five patients (38.5%) were WML positive, and 40 (61.5%) were WML negative.

As shown in Table 1, the duration RA was longer in the WML-positive group than in the WML-negative group. The resting systolic blood pressure, diastolic blood pressure, and heart rate were not significantly different between the 2 groups. Fasting plasma glucose was higher in the WML-positive group than the values in the WML-negative group. With regard to lipid metabolism, the concentration of serum triglyceride was higher but the concentration of serum HDL-C was lower in the WML-positive group than the WML-negative group. However, total cholesterol and LDL-C were not markedly different between the 2 groups. The levels of uric acid, creatinine, CRP, and ESR were similar between the 2 groups. The level of tHcy was higher in the WML-positive group than in the WML-negative group.

In univariate logistic regression analysis, the risk of WML was associated with duration of RA (odds ratio [OR], 1.01; 95% confidence interval [CI], 1.00-1.03; P = .0465), triglyceride (OR, 1.02; 95% CI, 1.00-1.04; P = .0369), HDL-C (OR, 0.91; 95% CI, 0.85-0.98; P = .0127), FPG (OR, 1.04; 95% CI, 1.01-1.08; P = .0127), and tHcy (OR, 1.35; 95% CI, 1.12-1.63; P < .0001) as the dependent lipid and glucose metabolic parameters in RA patients.

On the other hand, multivariate logistic analysis identified high tHcy (OR, 1.35; 95% CI, 1.12-1.63; P < .0001) in RA patients as the independent and significant risk factor for WML (Table 2).

Table 2
Multivariate logistic regression analysis with WML positive as the dependent variable in RA

	WML		
	OR	95% CI	P value
Homocysteine	1.35	1.12-1.63	<.0001

4. Discussion

In the present study, measurement of metabolic parameters revealed that serum HDL-C level was lower whereas triglycerides, FPG, and tHcy levels were higher in the WML-positive group than in the WML-negative group. Multivariate logistic analysis revealed that the high tHcy levels were independent risk factors for the presence of WML in RA patients.

Several studies have examined the prevalence of WML in both normotensive and hypertensive subjects. Shimada et al [24] studied 28 normotensive and 20 hypertensive patients aged 59 to 83 years and found the prevalence of advanced WML at 25% and 40%, respectively. Sierra et al [25] reported the prevalence of WML among asymptomatic never-treated hypertensive patients with mean age of 54 years at 40.9%.

The prevalence of WML was similar to the proportion of RA patients observed in the present study (25/65, 38.5%).

What is the pathophysiology of RA in relation to WML? Rheumatoid arthritis is a chronic inflammatory disease associated with mortality compared with the general population [2]. First, Ross [26] reported that inflammation plays an important role in the pathogenesis of atherosclerosis. Inflammation response injury is considered to be related to the initiation, growth, and complications of the atherosclerotic plaque [27]. Second, endothelial function has been impaired in RA patients [28]; and van Dijk et al [29] reported that endothelial dysfunction has been proposed as a causal mechanism of cerebral small-vessel disease.

There are several reports on the association between the presence of WML and tHcy [15-17]. The Rotterdam study found an association between tHcy and the presence of WML using qualitative measures [15]. Furthermore, a cross-sectional data by the Northern Manhattan Study provided evidence that tHcy was a risk factor for WML [16]. However, a prospective study by Dufouil et al [17] examined the relationship between tHcy and cognition decline in 1241 subjects aged 61 to 73 years; and they reported that WML did not mediate the association with homocysteine in healthy elderly people.

What are the mechanisms by which elevated tHcy levels lead to WML? In the authors' opinion, several mechanisms could explain this observation: First, elevated tHcy level in the blood induces oxidative injury to vascular endothelial cells and impairs the production of nitric oxide, which is a strong vascular relaxing factor, by the endothelium [30,31]. Second, hyperhomocysteinemia also enhances platelet adhesion to the endothelial cells [32], promotes growth of vascular smooth muscles cells [33], and is associated with higher levels of prothrombotic factors, such as β-thromboglobulin, tissue plasminogen

activator, and factor VIIc [34]. In addition, Hassan et al [35] reported that endothelial dysfunction has been proposed as a causal mechanism of cerebral small-vessel disease. Third, we have recently demonstrated that insulin resistance is associated with WML in patients with type 2 diabetes mellitus [36].

La Montagna et al [37] reported that insulin resistance is an independent risk factor for atherosclerosis in RA. In the present study, FPG was higher in the WML-positive group than the values in the WML-negative group; unfortunately, we did not measure fasting insulin concentration. Further investigations are needed to clarify whether insulin resistance is really involved in the relationship between WML and tHcy in RA patients.

The novel and important findings of the present study reveal that a greater proportion of the RA patients in the WML-positive group had greater tHcy than those in the WML-negative group.

There are several limitations to this study. Firstly, the present study was a relatively small size and was cross-sectional in design. Secondly, we did not measure nutritional status, that is, serum concentrations of folate and vitamins B_{12}, which could affect homocysteine metabolism [38]. Previous studies described that supplementation with folic acid increased the serum and red blood cell folate concentrations and decreased the plasma tHcy concentrations in healthy young Japanese male subjects [39]. Thus, it remains uncertain whether nutritional status could affect the presence of WML in RA patients. Finally, there are several studies that the levels of tHcy are related to age and renal dysfunction [10,11,40]. However, in our present study, the levels of tHcy between the WML-positive group and WML-negative group were not significantly changed by aging and/or circulating creatinine levels. It may be due to the mass of the study and/or other reasons. In any case, further investigations are needed to determine the relationships between aging, renal dysfunction, and the presence of WML in RA patients.

In conclusion, our findings suggest that the presence of WML is associated with elevated tHcy. The tHcy levels were independent predictors of WML in the Japanese patients with RA. In the future, large cohort studies including other populations may be beneficial.

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