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Factors Associated with Brain Atrophy Estimated with Automatic Voxel-Based Morphometry of Structural Magnetic Resonance Images in Elderly Diabetic Patients: Impact of Albuminuria on Hippocampal Atrophy

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Abstract

Background: We investigated what factors were associated with brain atrophy in elderly patients with type 2 diabetes.

Methods: We evaluated hippocampal and whole brain atrophy with automatic voxel-based morphometry of structural magnetic resonance image (MRI), voxel-based specific analysis regional analysis for Alzheimer's disease (VSRAD), in 70 diabetic subjects and 35 non-diabetic subjects. Cognitive function tests – MMSE, word recall (immediate and delayed), Digit Symbol Substitution test (DSST), and Stroop Color Word (Stroop) test were performed. Cerebral small vessel disease (SVD) was diagnosed as silent brain infarct and white matter lesions (WMLs) according to MRI.

Results: Significantly stronger hippocampal and whole brain atrophy were observed in diabetic patients than non-diabetic subjects. The levels of glycosylated hemoglobin A1c were significantly correlated with indices of hippocampal and whole brain atrophy. In diabetic subjects, hippocampal atrophy was independently associated with age, albuminuria, serum intercellular adhesion molecules -1 levels and lower diastolic blood pressure, while whole brain atrophy was associated with age and subcortical WMLs grade. Regarding an association between albuminuria and brain atrophy, significant hippocampal and whole brain atrophy were found in patients with albuminuria after adjusting for confounders. Hippocampal atrophy was independently associated with word recall and Stroop test after adjustment, while whole brain atrophy was also associated with word recall, DSST, and Stroop test, although the association weakened after adding degree of SVD to the variables.

Conclusions: Albuminuria was an independent risk factor for brain atrophy, especially hippocampal atrophy, which was associated with cognitive impairment, suggesting that the management of albuminuria may prevent progression of brain atrophy resulting in cognitive decline. In addition, the usefulness of VSRAD to support diagnosis of cognitive decline associated with brain atrophy was shown in daily clinical setting.

Keywords: Hippocampal atrophy; Albuminuria; Cognitive impairment; Diabetes mellitus

Introduction

The number of people with dementia is steadily increasing worldwide. Its complications can require huge expenditure for caregiving exceeding that for cancer and cardiovascular events and in addition, it places an immeasurable burden on caregivers. The number of older people with diabetes is also increasing as a result of increased average life expectancy and changes in lifestyle. In previous large-scale epidemiological studies, it has been reported that the incidence of dementia in diabetic patients is two- to threefold higher than in non-diabetic people [1,2]. Therefore, prevention of dementia in older people with diabetes will be one of the most important issues in diabetes treatment in the future.

Although many mechanisms have been considered for an association between diabetes and cognitive dysfunction [3,4], this has not yet been fully clarified. Generally, it is said that when accompanied by diabetes, progression to dementia is accelerated. Also, modest cognitive decrements have been reported to be already present at the early stage of type 2 diabetes [5]. Therefore, early diagnosis and countermeasures are needed. Although new neuroimaging techniques using positron emission tomography and single photon emission computed tomography have been recently developed in order to predict Alzheimer's disease, it is difficult to apply them in general clinical practice.

It has been reported that atrophy of the brain, especially the medial temporal lobe, is observed at a relatively early stage of Alzheimer's disease [6]. In addition, type 2 diabetes has consistently been associated with global brain atrophy [7]. Today, magnetic resonance image (MRI)

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is in widespread use and many trials have been conducted on using it to estimate brain atrophy. Further, with the method of evaluation using Voxel-Based Morphometry (VBM) brain atrophy can be easily semi-quantified. Matsuda et al. [8] developed a computer-assisted analysis using VBM for diagnosing Alzheimer's disease at an early stage. Free software for this procedure, called VSRAD (voxel-based specific regional analysis system for Alzheimer's disease), has made it possible to estimate hippocampal atrophy easily and speedily. It has been reported that hippocampal atrophy was stronger in diabetic patients as compared with non-diabetic people and the atrophy was associated with impaired cognitive function such as that indicated by MMSE [9,10]. However, with the first version of VSRAD, there were some difficulties in separating the gray matter of the brain, so it was insufficient for estimating the extent of whole brain atrophy. Recently VSRAD was improved by changing to statistical parametric mapping (SPM) 8 from SPM 2 and the use of diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL) for standardization greatly enhanced diagnostic accuracy for Alzheimer's disease and estimation of whole brain atrophy [11].

Therefore, the present study was conducted to investigate what factors were associated with hippocampal atrophy and whole brain atrophy assessed by VSRAD in fairly well controlled elderly patients with type 2 diabetes. In addition, we investigated an association between cognitive function domains and brain atrophy, and whether VSRAD is useful for providing supporting information for cognitive decline in the daily clinical setting.

Materials and Methods

We have observed cognitive function, MRI findings and clinical parameters in elderly patients with type 2 diabetes every three years since 2006 as previously reported [12,13]. The present study was conducted in 70 diabetic patients (mean age 76.6 ± 5.6 years old, 24 males and 46 females) adding to eight patients newly recruited in 2012. As control subjects, 35 age- and sex-matched non-diabetic subjects (mean age 75.3 ± 6.5 years old, 12 males and 23 females) were selected, who were outpatients with hypertension and/or dyslipidemia and underwent a health check-up. Patients with renal insufficiency (serum creatinine>2.0 mg/dl) and with history of stroke were excluded.

Cognitive function domains

We selected the following standardized psychological tests for measurement of each function as reported previously [12,13]. MMSE was used to assess orientation, registration, attention, calculation, language and recall with a score range from 0 to 30 [14]. We performed word recall, a subtest of the Alzheimer's disease assessment scale with a score ranging from 0 to 10, to assess verbal memory [15]. Immediately after the word list of 10 common nouns had been read, the respondents were asked to repeat as many words as they could recall in any order (immediate). After approximately 30 min of interference tasks, the respondents were asked to recall the 10-word list again (delayed). Complex psychomotor skills were evaluated by the Digit Symbol Substitution test (DSST), a subtest of the Wechsler Adult Intelligence Scale-Revised with a score ranging from 0 to 93 [16]. We used the modified Stroop Color Word (Stroop) test, consisting of two successive tasks, in order to assess attention/executive function. In the first task, the seconds to read 24 patches colored either in blue, yellow, green, or red were recorded and in the second task, the time to read the color of colored names printed in an incongruous color was recorded as quickly and accurately as possible. The task 1 - task 2 difference (Stroop score difference) evaluates attention and executive function. A wider time difference means lower cognitive performance [17]. Also, only MMSE was performed in non-diabetic subjects. All neuropsychological tests were administered by two well-trained psychological testers.

Evaluation of cerebral small vessel disease (SVD)

MRI was performed on a 1.5-T Signa Horizon system (GE Medical Systems, 1.5 Tesla, Milwaukee, WI). The imaging protocol consisted of T1-weighted spin-echo (inversion recovery; repetition time [TR/TE] = 2380/27.4 ms, matrix 320×224), T2-weighted fast spin-echo (TR/TE = 4017/103 ms, matrix 156×256), and fluid-attenuated inversion-recovery (FLAIR) (TR/TE = 8002/146 ms, matrix 256×192) sequences in the axial plane with a slice thickness of 5 mm and a 2 mm interslice gap. The slice thickness was 5 mm and interslice gap 2 mm. We defined silent brain infarction (SBI) as focal hyperintense areas larger than 3 mm in diameter detected on T2-weighted images, hypointense areas on T1-weighted images, and areas of higher intensity than cerebrospinal fluid in fluid-attenuated inversion recovery (FLAIR) images. Lesions less than 3 mm in diameter or with signal intensity similar to cerebrospinal fluid in FLAIR images were excluded because of the high possibility that they were enlarged perivascular spaces, even if they exhibited hyperintensity on T2-weighted images and a hypointensity on T1-weighted images. SBIs were divided into 3 categories depending on their number: 0, no SBIs; 1, 1-3 SBIs, and 2, >3 SBIs. The WMLs were stratified for periventricular and subcortical areas separately. The periventricular WMLs (PWMLs) and subcortical WMLs (SWMLs) were rated according to the Fazekas scale (PWMLs: grade 0, no lesions; grade 1, caps or pencil-thin lining; grade 2, smooth halo; grade 3, large confluence extending into deep white matter; and SWMLs: grade 0, no lesions; grade 1, punctate; grade 2, early confluent; grade 3, confluent) [18].

Automatic VBM of structural MRI (VSRAD)

The automatic VBM analysis was performed using the SPM 8 plus DARTEL based VBM analysis procedure as reported by Matsuda et al. [11]. The free software, VSRAD, is widely available in Japan. The analytical results automatically calculated by VSRAD are obtained through a comparison of the severity of gray matter atrophy in a Volume of Interest (VOI) at bilateral head to tail of the hippocampus, the entorhinal cortex, and amygdala regions and whole brain with its original normal database template.

Z-score maps for gray matter anatomical standardization and voxel normalization to global mean intensities (global normalization) were obtained using values calculated by the following equation; Z-score = ([control mean]-[individual value])/(control SD). The Z-score maps were displayed by overlay on tomographic sections and surface rendering of the standardized brain. The severity of atrophy in a target VOI was defined as the mean value of positive Z-scores in VOI (Hippocampal atrophy index, HAI). The extent of atrophy was calculated as the percentage of coordinates with a Z-score exceeding the threshold value of 2 in the whole-brain (whole brain atrophy rate, WBAR).

Evaluation of patients

Fasting blood samples were separated, and analyzed. High density lipoprotein (HDL)-cholesterol, triglycerides, low density lipoprotein (LDL)-cholesterol, and fasting blood glucose were measured using an autoanalyzer and routine enzymatic techniques. Glycosylated hemoglobin A1c (HbA1c) was measured using high-performance liquid chromatography (HLC-723G7, Tosoh, Tokyo, Japan), and was estimated as a National Glycohemoglobin Standardization Program

(NGSP) value (%), calculated by the formula HbA1c (%) = HbA1c (Japan Diabetes Society (JDS)) (%) x 1.02 + 0.25 [19]. Plasma c-peptide was measured by electrochemiluminescence immunoassay. Inflammatory markers were assayed using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay QuantiGlo kit (R&D Systems, Minneapolis, MN, USA) for tumor necrotizing factor-α (TNF-α) and interleukin-6 (IL-6) and immunosorbent assay kit (R&D Systems, Abingdon, Oxon, UK) for soluble intercellular adhesion molecule-1 (sICAM-1) as reported previously [20]. High-sensitivity C reactive protein (hs-CRP) was assayed using a monoclonal antibody coated onto polystyrene particles and fixed-timed kinetic nephelometric measurements (BN II; Dade Behring, Marberg, Germany). Adiponectin was assayed by latex turbidimetric immunoassay using Human adiponectin latex kit (Otsuka Pharmaceutical Co., Ltd. Tokyo, Japan). Apolipoprotein (Apo) E genotype was determined for each participant by the Invader assay [21]. The Apo E ε4 genotype was divided into two groups, one with ε4 (3/4 and 4/4) and one without ε4 (3/3 and 3/2).

We estimated glomerular filtration rate (eGFRcys) using Cystatin-C using the following three-variable Japanese equation: eGFRcys (mL/min/1.73 m^2) = (104×serum Cystatin-$C^{-1.019}$×0.996^{Age}× 0.929 (if female))-8, since eGFRcys has recently been proposed as being more suitable for estimating eGFR than creatinine, especially in elderly patients [22,23]. Decline in eGFRcys was defined as <60 mL/min/1.73 m^2. Albuminuria was defined as urinary albumin to creatinine ratio (ACR) ≥ 30 mg/g creatinine, calculated from two measurements of albumin (mg) and creatinine (g) in spot urine. Owing to the small number of participants with macroalbuminuria, albuminuria was not dichotomized into micro- and macro-albuminuria.

This study was approved by the ethics committee of Chubu Rosai Hospital. After informed consent was obtained from each of the participants, it was performed in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Results were expressed as mean ± SD. Statistical analysis was carried out using the t-test for comparisons between two groups. Comparisons for factors not forming normal distributions were carried out after log transformation. The chi square test was conducted for independence. We carried out multiple linear regression or multiple logistic regression where appropriate, and variables with $p<0.05$ in univariate analysis were entered for multiple regression analysis. Also, if two variables had collinearity, one of them was not entered. Statistical analyses were carried out using SPSS version 21 software (SPSS, Chicago, IL, USA), and $p<0.05$ was considered to be statistically significant.

Results

Clinical and biochemical characteristics of elderly diabetic patients stratified by median of hippocampal atrophy index (HAI) and whole brain atrophy rate (WBAR)

HAI was significantly higher in diabetic patients (average 1.72 ± 0.97, median 1.46, interquartile range; 1.06-2.11) as compared with non-diabetic subjects (1.37 ± 0.50, p=0.016). WBAR was also higher in diabetic subjects (average 9.51 ± 2.85%, median 8.75, interquartile range; 7.57-10.51) as compared with non-diabetic subjects (8.18 ± 2.71%, p=0.024). In biochemical and clinical backgrounds, significantly low levels of diastolic blood pressure and LDL-cholesterol were found in diabetic patients as compared with non-diabetic subjects (data not shown). The average levels of HbA1c were 5.75 ± 0.28%, ranging from 4.9 to 6.4% in non-diabetic subjects and 6.96 ± 0.71%, ranging from 5.7 to 8.9% in diabetic patients. Hemoglobin A1c level was significantly correlated with HAI, but not WBAR, in non-diabetic subjects, while such correlations with HAI and WBAR were not found in diabetic patients. In total subjects, HbA1c was significantly correlated with HAI and WBAR (Figure 1). After adjustment for age and sex, the correlation remained for HAI (p=0.021), while it was weakened for WBAR (p=0.075). Regarding cognitive function, MMSE was significantly lower in diabetic patients than in non-diabetic subjects (25.1 ± 3.2 vs 26.5 ± 2.4, p=0.035).

For diabetic patients alone, comparisons of two groups stratified by the median are shown in Table 1. Shorter education years, higher levels of uric acid and ACR, and higher frequency of albuminuria were observed in the high HAI group as compared with low HAI group, whereas higher age, higher levels of triglycerides, sICAM-1, TNF-α, IL-6, Cystatin C, and serum creatinine, higher eGFRcys, and higher frequency of albuminuria and eGFRcys decline were observed in the high WBAR group as compared with low WBAR group (Table 1). Regarding cognitive domains with brain atrophy, decreased levels of word recall and DSST were found in the high HAI group, whereas cognitive function domains except MMSE were worse in the high WBAR group as compared with the low WBAR group. Regarding SVD with brain atrophy, advanced SBI, PWMLs, and SWMLs were found in the high WBAR group, whereas there were no differences between the two groups for HAI.

Factors associated with HAI and WBAR

The Logistic analysis in Table 1, in which factors with $p<0.1$ were entered, showed that albuminuria (Odds ratio (ORs): 5.77, 95% confidence interval (CI): 1.67-19.89, p=0.006), and uric acid (ORs: 0.55, 95% CI: 0.35-0.88, p=0.012) were independent factors for the high HAI group, whereas only age (ORs: 6.14 per 10 years, 95% CI: 1.45-2.62, p=0.014) was an independent factor for the high WBAR group.

Regarding factors associated with HAI, univariate analysis showed that age, albuminuria, levels of sICAM-1, diastolic blood pressure, and smoking status were significantly correlated with HAI. Multivariate analysis showed that age, albuminuria, levels of sICAM-1, and diastolic blood pressure were independent factors. On the other hand, regarding factors associated with WBAR, univariate analysis showed that age,

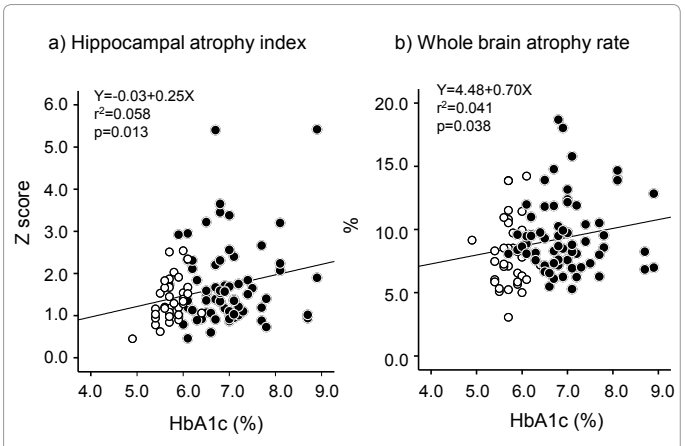

Figure 1: Correlation between HbA1c and hippocampal atrophy index (HAI) and whole brain atrophy rate (WBAR). Open circles are non-diabetic subjects and closed circles diabetic patients. HbA1c levels were correlated with neither HAI (r^2=0.003, p=0.745) nor WBAR (r^2=0.006, p=0.512) in diabetic patients, while they were correlated with HAI (r^2=0.416, p=0.013), but not WBAR (r^2=0.016, p=0.300) in non-diabetic subjects.

	Total	HAI (z score)		WBAR (%)	
		Low (<1.46)	High (≥1.46)	Low (<8.75)	High (≥8.75)
n (male/female)	70 (24/46)	35 (13/22)	35 (11/24)	35 (15/20)	35 (9/26)
Age (years)	76.6 ± 5.6	75.5 ± 5.0	77.6 ± 6.0	74.3 ± 4.3	78.8 ± 5.9****
Duration of diabetes (years)	20.6 ± 8.9	19.3 ± 9.4	21.9± 8.3	18.6 ± 8.2	22.6 ± 9.2#
Education years	9.59 ± 1.80	10.0 ± 1.9	9.0 ± 1.4*	10.1 ± 1.8	9.0 ± 1.6*
Smoking (-/+)	64/6	34/1	30/5	34/1	30/5#
Treatment (Diet/OHA/Ins)	14/45/11	5/26/4	9/19/7	7/25/3	7/20/8
Body mass index (kg/m^2)	23.4 ± 4.4	23.3 ± 3.8	23.6 ± 5.0	22.5± 3.6	24.3 ± 5.0#
Apo E ε4 (-/+)	61/9	30/5	31/4	31/4	30/5
Systolic BP (mmHg)	134.8 ± 17.6	136.3 ± 16.0	133.3 ± 19.0	134.5 ± 15.1	135.1 ± 20.0
Diastolic BP (mmHg)	71.6 ± 11.1	74.0 ± 9.8	69.2 ± 11.9#	72.8 ± 9.0	70.5 ± 12.9
HDL-cholesterol (mg/dL)	55.9 ± 15.5	55.3 ± 15.3	56.4 ± 16.0	57.8 ± 15.7	54.0 ±15.4
Triglycerides (mg/dL)	113.2 ± 55.7	112.4 ± 60.9	114.0 ± 50.9	95.5 ± 42.2	130.9 ± 62.2*
LDL-cholesterol (mg/dL)	103.2 ±25.4	103.3 ± 22.4	105.1 ± 31.3	101.2 ± 23.6	105.2 ± 27.2
FBG (mg/dL)	133.2 ± 30.8	136.2 ± 31.9	130.3 ± 29.7	134.2 ± 29.4	132.3 ± 32.5
HbA1c (%)	6.96 ±0.71	6.93 ± 0.67	6.98 ± 0.76	6.96± 0.75	6.95 ± 0.68
Fasting c-peptide (ng/mL)	2.08 ± 1.15	2.30 ± 1.21	1.85 ± 1.05	2.04 ± 1.16	2.11 ± 1.15
hs-CRP (mg/L)	1.06 ± 1.44	1.01 ± 1.52	1.11 ± 1.37	0.89 ± 1.31	1.23 ± 1.55#
sICAM-1 (μg/L)	218.1 ±80.8	209.2 ± 60.0	227.0 ± 97.3	190.8 ± 57.0	245.4 ± 91.9***
TNF-α (pg/mL)	1.96 ± 0.96	1.99 ± 0.90	1.93 ± 1.02	1.58 ± 0.73	2.34 ± 1.01****
IL-6 (pg/mL)	1.78 ± 1.57	1.64 ± 1.48	1.93 ± 1.67	1.49 ± 1.52	2.08 ± 1.59*
Adiponectin (μg/mL)	16.82 ± 11.41	16.51± 11.38	17.13 ± 11.59	16.57 ± 11.00	17.08 ± 11.96
Uric acid (mg/dL)	5.60 ± 1.45	6.01 ± 1.30	5.17 ± 1.48*	5.38 ± 1.15	5.82± 1.70
Creatinine (mg/dL)	0.84 ±0.26	0.87 ± 0.17	0.91 ± 0.32	0.70 ± 0.15	1.01 ± 0.26****
Cystatin C (mg/L)	1.11 ± 0.40	1.14 ± 0.43	1.08 ± 0.37	0.98 ± 0.22	1.25 ± 0.49***
eGFRcys (ml/min/1.73m^2)	64.8 ±22.8	63.0 ± 20.6	66.6 ± 24.9	71.6 ± 17.1	58.0 ± 25.7*
eGFRcys<60ml/min/1.73m^2 (-/+)	38/32	18/17	20/15	25/10	13/22***
ACR (mg/g creatinine)	54.5 ± 84.7	40.5 ± 59.8	68.6 ± 102.9*	37.4 ± 50.1	71.7 ± 107.0#
Albuminuria (-/+)	41/29	27/8	14/21***	25/10	16/19*
Cognitive test scores					
MMSE	25.14 ±3.21	25.60± 2.58	24.69± 3.72	25.34 ± 2.82	24.94 ± 3.60
Immediate word recall	6.44 ±1.54	6.81 ± 1.30	6.07 ± 1.69*	7.03 ± 1.06	5.85 ± 1.72***
Delayed word recall	6.47 ±2.41	7.14 ± 1.79	5.80 ± 2.77*	7.29 ± 1.95	5.66 ± 2.58***
DSST	32.62 ±11.10	35.86 ± 12.25	29.18 ± 8.66***	36.77 ± 11.56	28.21 ± 8.79*
Stroop	18.51 ±9.97	16.62 ± 7.85	20.47 ± 11.56	16.11 ± 7.79	20.99 ± 11.40*
MR findings					
SBI (0/1-3/3<)	30/25/15	14/14/7	16/11/8	20/9/6	10/16/9#
PWMLs (0/1/2/3)	26/31/11/2	14/15/6/0	12/16/5/2	19/12/4/0	7/19/7/2*
SWMLs (0/1/2/3)	20/36/12/2	12/17/6/0	8/19/6/2	14/17/4/0	6/19/8/2#

Values are expressed as mean ± standard deviation. #p<0.1, *p<0.05, **p<0.01, ***p<0.005, ****p<0.001; differences from low group in HAI and WBAR. Statistical analyses were carried out using the unpaired t-test for comparison of two means, and the chi square test for independence. Triglycerides, fasting c-peptide, high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor (TNF)-α, Interluekin-6 (IL-6), adiponectin, and albumin to creatinine ratio (ACR) were log transformed and comparison of the two means was carried out. BP, blood pressure; FBG, fasting blood glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, glycated hemoglobin; Apo E, apolipoprotein E; DSS, Digit Symbol Substitution; HAI, hippocampal atrophy index; WBAR, whole brain atrophy rate; OHA, oral hypoglycemic agent; SBI, silent brain infarction; PWMLs, periventricular white matter lesions; SWMLs, subcortical white matter lesions; sICAM-1, soluble intercellular adhesion molecule-1.

Table 1: Clinical and biochemical characteristics of elderly diabetic patients stratified by median of hippocampus atrophy index (HAI) and whole brain atrophy rate (WBAR).

duration of diabetes, treatment modality, SBI, SWMLs grade, PWMLs grade, IL-6, hs-CRP, sICAM-1, TNF-α, albuminuria, and eGFRcys decline were significantly correlated with WBAR. Multivariate analysis showed that age and SWMLs grade were independent factors (Table 2).

Association between brain atrophy (HAI and WBAR) and renal impairment

Since an association between brain atrophy and renal impairment - especially albuminuria - was observed, we investigated HAI and WBAR in the presence or absence of albuminuria or eGFRcys decline (Figure 2). HAI and WBAR were higher in patients with albuminuria than in those without. Significantly higher levels of HAI (p=0.004) and WBAR (p=0.019) in patients with albuminuria remained after adjustment for age and sex. The significant association remained after adjustment for education years, blood pressure, lipid levels, HbA1c, smoking status, and SVD grade in addition to age and sex (data not shown). WBAR, but not HAI, was also significantly higher in patients with eGFRcys decline. However, the significant difference between patients with and without eGFRcys decline disappeared after adjustment for age and sex. Regarding an association between brain atrophy and log ACR and eGFRcys, HAI had a tendency to be correlated with log ACR (r=0.210, p=0.081), and WBAR was significantly correlated with log

	Univariate analysis		Multivariate analysis	
	Correlation coefficient	p value	Standardized β coefficient (SE)	p value
HAI			r^2=0.395, p<0.001	
Age (years)	0.438	0.000	0.340 (0.017)	0.001
Albuminuria	0.347	0.003	0.213 (0.203)	0.044
sICAM-1 (μg/mL)	0.335	0.005	0.214 (0.001)	0.048
DBP (mmHg)	-0.304	0.011	-0.226 (0.009)	0.030
Smoking	0.249	0.037	0.128 (0.349)	0.212
WBAR			r^2=0.510, p<0.001	
Age (years)	0.516	0.000	0.402 (0.062)	0.002
SWMLs grade	0.411	0.000	0.269 (0.478)	0.045
Log IL-6 (pg/mL)	0.381	0.001	0.043 (1.272)	0.759
eGFRcys<60ml/min/1.73m^2	0.404	0.001	0.002 (0.749)	0.990
PWMLs grade	0.384	0.001	0.079 (0.462)	0.558
sICAM-1 (μg/mL)	0.348	0.003	0.086 (0.004)	0.425
Log TNF-α (pg/mL)	0.442	0.009	0.163 (1.762)	0.239
SBI	0.310	0.009	-0.141 (0.504)	0.321
Albuminuria	0.280	0.019	0.067 (0.625)	0.551
Duration of diabetes (years)	0.271	0.023	0.112 (0.035)	0.333
Log hs-CRP(mg/L)	0.271	0.024	0.071 (0.768)	0.580
Treatment modality	0.251	0.036	0.025 (0.523)	0.823

Smoking, no smoking: 0, smoking: 1, treatment modality, diet: 0, oral hypoglycemic agent: 1, insulin: 2. DPB: diastolic blood pressure. Variables with p<0.05 in univariate analysis were entered for multiple regression analysis.

Table 2: Factors associated with hippocampal atrophy index (HAI) and whole brain atrophy rate (WBAR).

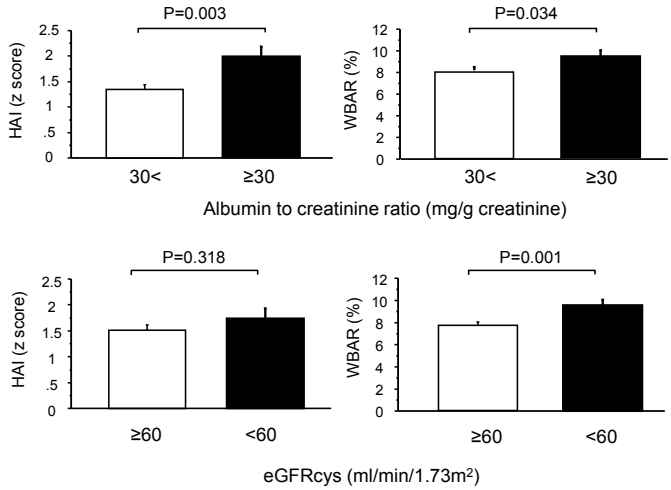

Figure 2: Comparison of hippocampal atrophy index (HAI) and whole brain atrophy rate (WBAR) in presence or absence of albuminuria or eGFRcys decline. Albuminuria was defined as albumin to creatinine ratio ≥ 30 mg/g creatinine and decline in eGFRcys was defined as eGFRcys<60 mL/min/1.73 m^2.

ACR (r=0.381, p=0.001) and eGFRcys (r=-0.435, p<0.001). However, the significant relationship also disappeared after adjustment for age and sex.

Associations between brain atrophy (HAI and WBAR) and cognitive function domains

HAI and WBAR were significantly correlated with each cognitive function domain. Associations of brain atrophy with individual cognitive function domains were examined in multivariate analysis. HAI was independently associated with word recall and Stroop test and the association remained after adjustment for additional variables. WBAR was independently associated with word recall, DSST, and Stroop test after adjustment. However, the association with DSST weakened after adding degree of SVD to the variables (Table 3).

Discussion

A large number of studies have shown that a smaller brain volume due to atrophy, especially hippocampal atrophy, on MRI was noted in subjects with Alzheimer's disease or mild cognitive impairment as compared with those without, and such atrophy was associated with cognitive decline and incident dementia [6]. It is well known that impaired cognitive function has been found in diabetic patients, as compared with non-diabetic subjects, in large epidemiological studies [1,2] and it has also been reported that brain atrophy was greater in diabetic patients than in non-diabetic subjects [7,9,10]. The findings of the present study were in agreement with many previous studies. However, the mechanisms by which greater brain atrophy occurs in diabetic patients have not been fully resolved.

Therefore, we investigated what factors were associated with brain atrophy in elderly diabetic out-patients who were relatively well controlled according to the diabetic treatment guidelines and found that age, diastolic blood pressure, albuminuria, and sICAM-1 levels were independent factors for hippocampal atrophy, and age and SWML grade for whole brain atrophy.

In particular, it is noteworthy that greater hippocampal and whole brain atrophy were found in patients with albuminuria as compared with those without, and the relationship remained significant after adjusting for confounders in the present study. In contrast, though an association between eGFR decline and whole brain atrophy, but not hippocampal atrophy, was also found, the relationship disappeared after adjusting for age and sex.

So far, many studies have shown that albuminuria was associated with cerebral SVD [24,25] and cognitive impairment [26]. We also reported an association between albuminuria and cognitive impairment in our cross-sectional and longitudinal study in elderly diabetic patients [27,28]. In other research, we reported that advanced SVD was associated with cognitive decline in elderly diabetic patients [13,29]. A few studies have investigated an association of albuminuria and brain atrophy [30,31]. Although Sink et al. [32] have recently also reported that ACR was significantly associated with higher WML volumes and greater atrophy in African Americans with type 2 diabetes, they found no significant association of either ACR or eGFR and hippocampal atrophy.

Generally, since the brain and kidney could be considered as end organs having similar low-resistance vascular beds and endothelial structures, impaired endothelial function in the brain could lead to SVD through damage to the blood-brain barrier, whereas endothelial dysfunction in the kidney might contribute to impaired glomerular filtration and secondary protein leakage [33]. Albuminuria is a measure for endothelial pathology and we reported previously that levels of sICAM-1, which reflect endothelial damage, were associated with the presence and progression of SVD [20], and cognitive decline [29]. In the present study, sICAM-1 levels were also an independent factor for hippocampal atrophy. Taken altogether, it is likely that endothelial impairment is a risk factor for SVD and brain atrophy, leading to cognitive impairment. Indeed, an association between SVD and brain atrophy has been often reported [34]. However, the present

	MMSE	Word recall		DSST	Stroop test
		Immediate	Delayed		
HAI					
Univariate analysis	-0.284*	-0.470****	-0.470****	-0.311*	0.278*
Multivariate analysis					
Model 1	-0.229(0.409)#	-0.367(0.185)***	-0.440(0.298)***	-0.210(1.498)	0.300(1.375)*
Model 2	-0.065(0.544)	-0.406(0.246)**	-0.503(0.390)***	-0.189(1.689)	0.445(1.692)**
Model 3	-0.116(0.567)	-0.459(0.250)**	-0.571(0.399)***	-0.214(1.708)	0.486(1.766)**
WBAR					
Univariate analysis	-0.263*	-0.494****	-0.463****	-0.492****	0.361***
Multivariate analysis					
Model 1	-0.191(0.149)	-0.396(0.067)***	-0.459(0.108)***	-0.442(0.505)***	0.453(0.482)***
Model 2	-0.057(0.177)	-0.366(0.081)*	-0.453(0.128)***	-0.332(0.526)*	0.372(0.560)*
Model 3	-0.071(0.197)	-0.350(0.090)*	-0.522(0.141)**	-0.270(0.582)#	0.485(0.612)**

In the multivariate analysis cognitive function domains were used as dependent variables. Values are correlation coefficients in the univariate analysis and standardized β coefficient (SE) in the multivariate analysis. Model 1 is adjusted for age, sex, and years of education. Model 2 is adjusted for duration of diabetes, body mass index, current smoking, treatment modality, diastolic blood pressure, log triglycerides, uric acid, sICAM-1, log hs-CRP, log TNF-α, log IL-6, albuminuria, and eGFRcys<60ml/min/m^2 in addition to the factors in Model 1. Model 3 is adjusted for SBI, PWMLs and SWMLs in addition to the factors in Model 2. #p<0.1, *p<0.01, **p<0.01, ***p<0.005, ****p<0.001

Table 3: Associations between hippocampus atrophy index (HAI) and whole brain atrophy rate (WBAR) and cognitive function domains.

study showed no clear relationship between brain atrophy and SVD, though a partial association was observed between SWML grade and whole brain atrophy.

On the other hand, regarding cognitive domains, it has been reported that SVD was mainly associated with cognitive dysfunction in the frontal lobe [35]. In our previous longitudinal study, impairment of executive functioning and processing speed, which were reflected in the DSST and Stroop test, were found in patients with progression of SVD [13,29]. In contrast, a recent 3-year longitudinal study showed a strong association between progression of albuminuria and a decline in verbal memory [28]. Although most studies have found little evidence that brain atrophy specifically contributes to memory deficits in diabetes [7], our results showed that brain atrophy, especially hippocampal atrophy, was strongly associated with word recall, especially delayed word recall, rather than DSST and Stroop test, suggesting that neurodegeneration related to brain atrophy might cause cognitive decline by a mechanism different from that for SVD.

Verdelho et al. [36] reported that vascular dementia was predicted by previous stroke and white matter changes, that is cerebrovascular injury, while Alzheimer's disease was predicted only by medial temporal atrophy. In contrast, Moran et al. [37] recently reported that neurodegeneration as a result of brain volume loss rather than cerebrovascular lesions might play a key role in type 2 diabetes-related cognitive impairment. Zlokovic [38] hypothesized in his review that hypoperfusion-hypoxia resulting from decreased cerebral blood flow and decreased clearance of β amyloid and accumulation of neuro-toxic substances resulting from blood-brain barrier damage caused by endothelial dysfunction might be factors of cognitive impairment due to vascular factors. Since in most cases Alzheimer-type and vascular pathologies co-occur [39], such a mechanism is likely to be involved. Therefore, further study will be needed on the etiological significance of diabetes-associated brain atrophy and the related cognitive impairment.

Regarding the other factors, diastolic blood pressure was independently negatively associated with hippocampal atrophy. Different effects of mid- and late-life blood pressure on the brain have been considered [40] and it has been reported that a decrease in diastolic blood pressure was associated with brain atrophy [41]. In the present study, since about 70% of the study subjects were treated with renin-angiotensin system inhibitors and the averages of systolic and diastolic blood pressure were less than 140 and 80 mmHg, respectively, attention should be paid to severe decreases in diastolic blood pressure with strict control of blood pressure in brain atrophy.

While it is well known that poor glycemic control accelerates brain atrophy [42], our study found no relationship between these factors in diabetic patients. The reason for this might be heterogeneity of diabetes, since HbA1c levels were significantly correlated with hippocampal atrophy in total subjects and in non-diabetic subjects.

Regarding an association with inflammatory markers, it has been reported that hippocampal atrophy estimated with VSRAD was associated with hs-CRP [43] and adiponectin levels [44]. Associations of hs-CRP and adiponectin with hippocampal atrophy were not found in the present study. However, since it has been reported that markers of low-grade inflammation and endothelial dysfunction were related to cognitive impairment [45], it is likely that inflammatory changes might have some effects on brain atrophy.

Another characteristic finding of our study was that hippocampal and whole brain atrophy estimated with VSRAD were well correlated with a variety of cognitive function indicators - decreased word recall for hippocampal atrophy and worsened DSST and Stroop test in addition to word recall for whole brain atrophy. However, the free VSRAD software was developed as a tool for VBM using data from a Japanese database so if it is going to be used for people of other ethnic groups, it will be necessary to modify the software to suit databases for such people [46]. Although VBM comparison methods also require spatial normalization and registration of the brain to a standard template, simple and easy-to-use tools like VSRAD should provide useful information regarding diagnosis of Alzheimer's disease and future decline in cognitive function in the everyday clinical setting. Now, we are investigating whether brain atrophy estimated with VSRAD can predict cognitive decline.

Several limitations of this study should be taken into consideration. It was a small-scale hospital-based cross-sectional study and treatment according to the guidelines may have influenced the results. Also, cognitive impairment due to vascular cognitive impairment was indistinguishable from that due to Alzheimer's disease.

In conclusion, albuminuria was an independent risk factor for brain atrophy, especially hippocampal atrophy, which was associated with cognitive impairment, suggesting that management of albuminuria at

the early stage may prevent progression of brain atrophy resulting in cognitive decline. In addition, in the daily clinical setting the usefulness of VSRAD in supporting diagnosis of cognitive impairment associated with brain atrophy was demonstrated.

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