adjacent to lateral ventricles. There was no association between clinical pictures of diabetics with cognitive dysfunction and of those with morphological changes in the brain.

Conclusions. Impaired cognitive domains of speed of mental processes and memory were associated with WMHs and subcortical atrophy. Degenerative changes in cerebral small vessels may constitute predictive factors for the rate of cognitive dysfunction in elderly diabetics.

Keywords: Type2 diabetes mellitus, Elderly, Cognitive dysfunction, Periventricular hyperintensity, White matter hyperintensities, Subcortical atrophy.

Introduction

Type 2 diabetes is an age-related disease with a prevalence in Japan estimated at more than 5 percent of the population (1). For elderly diabetics, the purpose of treatment is not only to control plasma glucose levels, but also to prevent diabetic complications. Prospective intervention studies have provided evidence that intensive glycemic control effectively slows the onset and progression of diabetic vascular complications associated with type 2 diabetes (2). However, these epidemiological investigations did not consider the various aspects to prevention of cognitive decline in elderly diabetics.

The influence of diabetes on brain function has been of interest for more than 80 years (3, 4). Subjects with type 2 diabetes initially manifest deficits in abstraction, problem solving, memory and the completion of tasks involving speed and complex perceptual-motor responses. Recently, several epidemiological studies have shown that diabetes increases the risk for the most common forms of dementia, Alzheimer's disease and vascular dementia (5-9). Hence, the most critical issue is to identify the factors responsible for diabetic cognitive impairment that lead to severe cognitive decline in the elderly. Diabetes-related brain disorders have been considered multifactorial and attributed to genetic predisposition, nutritional factors, cerebrovascular disorders, and the neurotoxic effects of hypoglycemia and hyperglycemia (10).

The so-called Rotterdam study, which is one of the largest population-based cohort studies, demonstrated conclusively that diabetic subjects with cerebrovascular diseases and with insulin treatment are more prone to dementia (5). Recent biological findings have supported the view that several risk factors could be linked between diabetes and cognitive dysfunction in the elderly (10, 11). However, clinical pictures of elderly diabetes are vary and elderly diabetics may have coincident neuropsychiatric disorders, thus making it difficult to identify the factors specifically responsible for cognitive decline.

To address these controversies regarding cognitive decline in elderly diabetics, we conducted a large-scaled prospective study of the Japanese Elderly Diabetes Intervention Trial (J-EDIT). J-EDIT was a prospective intervention study designed to investigate and identify the clinical characteristics of non-demented diabetic elderly. In the report presented here, we have analyzed the baseline measures of cognitive dysfunction in non-demented elderly with type 2 diabetes. The aim of this study was to explore possible associations among diabetic cognitive dysfunction, brain morphological changes detected on magnetic resonance (MR) imaging, and diabetic clinical features. To analyze brain MR images, we focused on white matter hyperintensities (WMHs) and subcortical brain atrophy because subcortical structural changes have been associated with cognitive impairment in demented and nondemented elderly

subjects (12, 13). We classified hyperintensities into periventricular, deep white matter, thalamic and basal ganglia. The research questions were: 1) What diabetic indices are associated with cognitive dysfunction? : 2) Which WMHs influence specific cognitive domains of elderly diabetics? : 3) Do brain structural changes on MR imaging correlate with clinical measurements of diabetes? For this study, we adopted the canonical correlation analysis to address these questions.

Materials and Methods

Participants: J-EDIT started in 2001 as a prospective intervention study of Japanese elderly with diabetes mellitus to prevent the several diabetic complications. The study involved 1,173 diabetic subjects from 42 institutes and hospitals in Japan who were 65 years or older (mean age was 71.8 $\pm$ 4.6) and whose serum HbA1c levels were $\geqq$ 7.0%. Written informed consent was obtained from all patients. From these subjects enrolled in the J-EDIT we selected 95 subjects with type 2 diabetes (14), who were treated at Kobe University Hospital, Nagoya University Hospital, Chiaki Hospital, Aoki Memorial Hospital, Nagoya Kyoritsu Hospital and Tokyo Metropolitan Geriatric Hospital. The diabetic participants who had difficulties in communicating, or showed signs of speech disturbance, deafness, severe disturbance of visual acuity, dementia,

and serious deterioration of the activities of daily life were excluded from this study. Clinical diagnosis of dementia was established according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV (15). Subjects with chronic renal failure (serum creatinine > 1.5 mg/dl), serious heart failure or symptomatic cerebral infarctions were also excluded from this study.

Assessment of diabetes mellitus, complications, and comorbidities: The diagnosis and information of diabetes mellitus, blood examinations and complications were obtained from clinical charts (14). Blood samples were obtained by vein puncture after overnight fasting to assess serum levels of glucose, HbA1c, total cholesterol, triglyceride, and HDL-cholesterol. Serum insulin concentrations were measured in patients who were not receiving insulin therapy. Diabetic vascular complications were assessed for the co-existence of nephropathy, retinopathy, neuropathy and coronary diseases. Retinopathy was assessed fundoscopically through the dilated pupils by experienced ophthalmologists. The degree of retinopathy was classified into five categories; 0 (no retinopathy), 1 (intra-retinal hemorrhages and hard exudates), 2 (soft exudates), 3 (intra-retinal microvascular abnormalities, venous calibre abnormalities and venous beading), 4 (neovascularization of the disc or elsewhere in the retina, preretinal fibrous tissue proliferation, preretinal or vitreous hemorrhage, and retinal detachment). Nephropathy was assessed in

terms of the mean urinary albumin-to-creatinine ratio (ACR) and rated as 1 (no nephropathy: ACR<30 μg/mg), 2 (microalbuminuria: 30≦ ACR< 300 μg/mg), or 3 (persistent proteinuria: ACR≧ 300 μg/mg or urinary protein≧ 30 mg/dl). Diabetic neuropathy was classified as 1 (no neuropathy), 2 (loss of Achilles tendon reflex without neuropathic symptoms including paresthesia), or 3 (neuropathic symptoms). Coronary artery diseases were considered to be present when diabetic patients had at least one of the following: a history of myocardial infarction characterized by a typical clinical picture (chest pain, chest oppression, dyspnea, typical changes on ECG accompanied by pathological Q waves and/or localized ST variations), and typical enzymatic changes. Because we adopted canonical correlation analysis for this study, we had to minimise the number of the clinical variables. To assess the global diabetic microvascular complications by a single variable in each of the diabetic patients, we calculated the diabetic microvascular complication score. (Microvascular complication score = 0.51* retinopathy + 0.54* neuropathy + 0.67* nephropathy). Microvascular complication score statistically reflected the general features of the diabetic microvasculopathies in this study. Cardiovascular complications were classified into two categories, that is, presence or absence of coronary artery diseases.

Clinical diagnosis of hypoglycemia was based on the modified Whipple triad: symptoms and/or signs consistent with a low glucose concentration, low plasma glucose concentrations (< 60 mg/dl), and relief of symptoms associated with restoration of plasma glucose level (16). Subjects with at least one hypoglycemic episode during the recent twelve months were considered to have hypoglycemia.

Procedures for analysis of the brain MR imaging: For every diabetic subject, a series of axial standard T1-weighted (repetition time [TR], 400msec; echo time [TE], 12msec), T2-weighted (TR, 3000msec; TE, 90msec; a 256 x 512 matrix) and fluid-attenuated inversion-recovery (FLAIR) (TR, 7500msec; TE 110msec; inversion time, 2200msec; a 256 x 512 matrix) MR sequences of the brain were performed using 1.5 Tesla MR units (Gyroscan NT-Intera and Gyroscan ASC-NT, both Philips, Eindhoven, The Netherlands; SIGUMA MR/I, General Electric, Milwaukee, WI). Scans in parallel with the anterior commissura – posterior commissura line were performed from the vertex to the foramen magnum with 7 mm-thick slices and an inter-slice gap of 1.4 mm.

We analyzed WMHs and subcortical brain atrophy on MR images. WMHs appeared as hyperintense on T2 weighted images, but did not leave a clear hypointense hole on T1 weighted images (Figure 1 left). FLAIR was used to obtain a clearer picture of the various WMHs (17), which were classified into

subcortical WMHs and periventricular hyperintensity (PVH) (Figure 1 right). WMHs were considered periventricular if the largest diameter was adjacent to the ventricular lining; otherwise, they were considered subcortical. PVH was rated semiquantitatively as 0 (none), 1 (pencil thin lining: <3 mm from the edge of ventricles), 2 (smooth halo: 3-10 mm), 3 (extending cap or thick lining: 10-25 mm), 4 (large confluent: >25 mm) for three separate regions; adjacent to frontal horns (frontal caps), adjacent to the wall of the lateral ventricles (bands), and adjacent to the occipital horns (occipital caps). The overall degree of PVH was calculated by adding up the scores for the three separate compartments (range 0-24) (18, 19). The number and size of subcortical WMHs were counted in the frontal, parietal, occipital, and temporal lobes, as were the number and size of hyperintensities in the basal ganglia and thalamus. The size of subcortical WMHs was classfied, according to the largest diameter, that is, small (1-3 mm), medium (3-10 mm), or large (>10 mm) (17). To calculate the volume of subcortical hyperintensities they were assumed to be spherical with a fixed diameter of 2, 6, 12 mm for each of the three respective categories.

Linear analysis of subcortical brain atrophy, Evans Ratio (ER), inverse Cella Media Index (iCMI), Caudate Head Index (CHI), and Basal Cistern Index (BCI) were all calculated (20-23). The following were measured with slide calipers: the maximum distance between the tips of the anterior horns (A), the width between

the bilateral heads of the caudate nuclei (B), the maximum transverse inner diameter of the intracranial space (C) , the maximum width of the cella mediae (D) and the maximum transverse inner diameter (E). Finally, the internal width between the bilateral temporal lobe (F) and the maximum transverse inner diameter (G) were calculated. The CHI, iCMI, ER and BCI were calculated with the following respective formulae: CHI=B/C, iCMI=D/E, ER=A/C and BCI=F/G, respectively (Figure 2).

Two raters who had no knowledge of the clinical data analyzed the brain MR imaging. To test the interrater reliability, the result of the two raters was subjected to correlation analysis for comparison in a random sample of 15 subjects. The analysis showed a strong correlation ($r=0.85–0.89$, $P<0.0001$), which suggested that the method of measurement used for this study was reliable.

Measurement of Cognitive Function: We used neuropsychiatric test batteries to examine cognitive function of each of the subjects by assessing the speed of cognitive processes, as well as the extent of verbal memory, and global cognitive function. Two tests were used to assess the speed of mental processes: an abbreviated Stroop tests (reading color names), and the digit symbol substitution test of the Wechsler Adult Intelligence Scale-Revised (24, 25). Verbal memory was assessed with the immediate and delayed word-list

recall from the logical memory subtest of the Alzheimer's Disease Assessment Scale and of the paragraph from the neuropsychological tests of the National Center of Neurology and Psychiatry, Japan (26, 27). Finally, MMSE was used to measure global cognitive function (28).

Statistical analysis: To explore the association among cognitive profile, clinical variables of diabetes, and morphological changes in MR imaging, we used the Pearson correlation and canonical correlation analyses (29). There were four sets of variables: seven cognitive tests, sixteen clinical variables of diabetes, eight brain WMH regions and four indices of brain atrophy. A total of 196 Pearson product moment correlations were identified between changes in cognitive tests and other measures. Since multiple test corrections would wipe out virtually any test significance, and variables within the same class are often showed a high degree of correlation, thus rendering the correlations redundant, we adopted the canonical correlation analysis to identify the direct relationship between two sets of variables. Canonical correlation analysis is essentially a Pearson correlation between the linear combination of variables in one set and the linear combination of variables in another set. The pair of linear combinations with the highest correlation is determined first. The pairs of linear combinations are known as canonical variables and their correlations as canonical correlations. The first canonical variables have the highest

explanatory power. The sign and magnitude of the correlation coefficients among MR imaging findings, cognitive function and clinical indices indicate the relative direction and strength of the correlation. The study reported here is the first to establish canonical correlations and their statistical significance expressed in the P values. Statistical significance was defined as $p < 0.05$.

Results

Clinical characteristics of the 95 elderly diabetic patients are shown in Table 1. The mean age of the patients and duration of diabetes mellitus were 72.8±0.5 years and 18.5±1.2 years, respectively. Average HbA1c was 7.9±0.5%, while other indices, such as blood pressure, serum cholesterol level and body mass index, were within reasonable limits. Eighteen diabetic subjects were receiving insulin therapy and 21 patients had hypoglycemic episodes. Because the number of patients in this study with insulin treatment or with hypoglycemic episodes was small, we could not analyse the effect of insulin therapy or hypoglycaemia on cognitive dysfunction.

Table 2 shows the Pearson correlation coefficients for cognitive profiles and other measurements. Age correlated positively and educational background negatively with diabetic cognitive dysfunction, particularly with impaired word-list recall and the paragraph recall. Subcortical brain atrophy indices including ER,

CHI, and iCMI were also related to immediate word-list recall and digit symbols substitution test results. However, we could not detect any correlation between diabetic cognitive impairment and WMHs on MR image (data not shown). Canonical correlation analysis indicated a strong association of cognitive function with WMHs (p=0.004) (Table 3). The canonical valuables of WMHs was contributed with digit symbol substitution test, MMSE and delayed word-list recall (correlation of canonical variables: 0.48, 0.39, 0.32, respectively) and the canonical valuables of cognitive function was negatively contributed with PVH and WMHs in the temporal and parietal lobes and in the thalamus (correlation of canonical variables: -0.32, -0.23, -0.21, -0.21, respectively), but not with WMHs in frontal lobe. In other words, digit symbol substitution test, MMSE and delayed word-list recall had a negative correlation with PVH and with WMHs in the temporal and parietal lobes and in the thalamus. These results suggest that diabetic patients with more predominant WMHs, particularly in periventricular areas, were more deficient in speed of mental processes and in verbal memory. Table 4 shows the relationship between cognitive function and clinical indices. No correlation could be established between cognitive function and clinical indices of diabetes, including glycemic control, lipid metabolism, blood pressure and complications of diabetes mellitus (p=0.881). Table 5 makes it clear that

there was no correlation between WMHs and diabetic clinical pictures including diabetic control and complications (p=0.500).

Table 6 shows a clear correlation of diabetic cognitive dysfunction with subcortical brain atrophy indices (p=0.010). Lower scores for word-list recall and digit symbol substitution test, as well as delayed paragraph recall and extended Stroop A were positively associated with enlarged ER, CHI and iCMI. Canonical correlation did not detect a significant association between brain atrophy and diabetic clinical indices (p=0.999) (Table 7).

Discussion

The study presented here represents the first investigation analyzing the possible associations between cognitive dysfunction and clinical features, with simultaneous evaluation of brain morphological changes detected on MR images in nondemented elderly with type 2 diabetes. It was found that periventricular WMHs and subcortical brain atrophy strongly correlate with several domains of diabetic cognitive impairment, such as impaired speed of cognitive processes and memory. Our study also indicates that the various and separate subcortical WMHs in the parietal and temporal lobes and in the thalamus, but not in the frontal lobe, are associated with diabetic cognitive impairment. However, we could not detect the diabetic factors responsible for

cognitive dysfunction, nor for the morphological changes on MR images, in spite of our thorough investigation of the various diabetic indices, including diabetic control, complications and comorbidities. These findings were established with the canonical correlation analysis used for our study.

Previous studies have investigated the relationship between WMHs and cognitive decline in non-demented and demented elderly (18, 30-34). Cognitive test scores in older adults were found to be worse in the presence of severe WMHs, even after adjustments for age, gender and education (18). PVH is more likely than subcortical WMHs to be associated with speed of cognitive processes and memory (18, 30, 31). The white matter of the subcortical structure can be divided into the area just beneath the neocortex and the area surrounding the ventricles. The periventricular region contains many long association fibers that connect the cerebral cortex with subcortical nuclei such as found in the striatum and with more distant cortical areas. On the other hand, the subcortical region close to the neocortex features a high density of short looped U-fibers connecting adjacent cortical areas (35). Periventricular WMHs damage the long-tract white matter pathways connecting many cortical areas, which might explain their profound effects on multiple domains of cognition.

We had expected that diabetic cognitive dysfunction is likely to be associated with WMHs in the frontal lobe, because the executive/frontal lobe function is

often impaired in diabetics. Besides PVH, however, impaired cognitive function in elderly diabetics was correlated with subcortical WMHs in parietal and temporal lobes as well as in the thalamus. Recent neuroradiological studies have demonstrated reduced regional cerebral blood flow and metabolism in Alzheimer's diseases especially in the parieto-temporal cortical areas and correlated with the distribution of Alzheimer pathological features, while the primary sensori-motor and visual cortical areas were relatively preserved (36, 37). Symptoms of early stage Alzheimer's disease originate from the impaired temporal and parietal lobe functions. Incidental onset of Alzheimer's disease in elderly diabetics can be expected to exacerbate the functional loss and clinical symptoms related to temporal and parietal lobes, as also observed in the case of diabetic cognitive dysfunction.

The pathophysiological origins of WMHs are still unclear, with vascular and nonvascular contributions likely to be causative factors. Subcortical WMHs on T2 weighted MR imaging correlate with several pathological changes such as myelin pallor, dilatation of the perivascular space, myelin or axonal loss, scattered cystic infarcts and angionecrosis. Periventricular hyperintensities on MR scans are associated with partial break down of the ependymal cell lining and subependymal gliosis in addition to the pathological changes of subcortical WMHs (38, 39). Although characteristic pathological features of the diabetic

brain have yet not been identified, vascular compromise is common in the elderly and is accompanied by damage to white matter pathways (12, 40). Age and hypertension have been the most consistent predictors of WMHs (32, 41-43), while some other studies have indicated that diabetes increases the risk of WMHs (13, 32, 44). The increased WMHs in elderly diabetics, presumably accompanied by as yet unidentified clinical variables, may account, at least in part, for diabetic cognitive dysfunction.

To reduce the rate of dementia in elderly diabetics, it is crucial to identify the factors responsible for the progression towards severe cognitive decline. Degenerative changes in cerebral small vessels may affect diabetic cognitive dysfunction, while it seems likely that it is also influenced by diabetic metabolic abnormalities and complications with or without unidentified genetic susceptibility. However, the results of our study do not support the notion of any relationship between cognitive dysfunction and diabetic clinical characteristics. Whether variations in glucose homeostasis influence cognitive function remains controversial (45-47), although it has been found that the cognitive function of diabetic and nondiabetic subjects fluctuates in accordance with the serum glucose levels (46, 47). At the same time, increased insulin resistance is associated with atrophy of medial temporal lobe structures in elderly diabetics (48). For these reasons, new surrogate markers that reflect chronic

hyperglycemia in the diabetic brain are needed. Hyperglycemia causes oxidative stress via the polyol pathway, enhances advanced glycation end-products (AGE), and increases lipid peroxidation and imbalances in the generation of reactive oxygen species and their scavengers (49, 50). N^{ε}-Carboxymethyllysine (CML), the most prominent AGE product, is crucially involved in the development of diabetic microangiopathy (51), and the level of CML expression is high in the blood vessels and brain of diabetics, but low in aging controls (52). Oxidative stress has also been implicated in the pathophysiology of Alzheimer's disease and hypoxic brain insults (53-55). Progression of diabetic retinopathy strongly correlates with the total sum of blood glucose control, and may constitute a predictor for cerebral small vessel disease (56, 57).

Certain limitations of our study need to be considered. The first limitation is that this analysis was a cross-sectional study. The second is the possibility that participants with some other cognitive dysfunction could be involved in this study. The mean MMSE score of our diabetic patients was 26.2 ± 0.3, which means that most of the subjects did not attain the full MMSE score. In particular, we could not completely exclude the patients with mild cognitive impairment. The third limitation is the method for measuring brain atrophy. We used linear measurements to evaluate subcortical atrophy adjacent to the lateral ventricles,

and this procedure is outdated and less accurate than the recently developed volumetric analysis of MR images (58). However, volumetric MR analyses of a number of diabetic patients from the different institutes and hospitals could not be performed. Finally, we did not estimate the effects of treatment per se for diabetes, hypertension, and lipid abnormalities on diabetic cognitive domains. The Rotterdam study has suggested that use of oral medication and insulin treatment for diabetes increases the association with dementia. While MR imaging has demonstrated that hypertension is associated with cognitive dysfunction and WMHs (59, 60). Recent prospective intervention studies have indicated that appropriate blood pressure control delays the progression of cognitive decline (61). Furthermore, there are indications that impaired cholesterol transport may have a pathophysiological roles in Alzheimer's disease and that HMG-CoA reductase inhibitors (statin) may have a protective effects on cognitive dysfunction in the elderly (62-64). Most of the diabetic subjects enrolled in this study were successfully treated for their blood pressure and lipid abnormalities, which could diminish the impacts of hyperglycemia, hypertension, and lipid abnormalities on diabetic cognitive impairment and brain structural changes. The effects of these limitations of our study will be analyzed in the prospective intervention J-EDIT study.

In summary, WMHs and subcortical brain atrophy observed on MRI scans of elderly diabetics without symptomatic brain infarctions were found to be associated with impaired speed of mental processes and memory, while WMHs are thought to be responsible for degenerative changes of cerebral small vessels and to be implicated in the pathogenesis of cognitive impairment. These findings suggest that periventricular WMHs and subcortical atrophy in particular constitute predictors of the rate of cognitive dysfunction in elderly diabetics and may underlie procession towards severe cognitive impairment. Our prospective J-EDIT study should help to determine the factors that can prevent cognitive dysfunction in elderly diabetics.

Acknowledgements

This work was supporeted by a Research Grant for Longevity Sciences from the Ministry of Health, Labour and Welfare,Japan, and a grant from the Novartis Foundation for Gerontological Research (T.S.).

References

1. Kawamori R. Diabetes trends in Japan. *Diabetes Metab Res Rev.* 2002; 18: S9-S13

2. Davidson JA. Treatment of the patient with diabetes: importance of maintaining target HbA(1c). *Curr Med Res Opin.* 2004; 20: 1919-1927

3. Miles WR, Root HF. Psychologic tests applied to diabetic patients. *Arch Intern Med.* 1922; 30: 767-777

4. Strachen MW, Ewing FM, Deary IJ, et al. Is type 2 diabetes associated with an increased risk of cognitive dysfunction? A critical review of published studies. *Diabetes Care* 1997; 20: 438-445

5. Ott A, Stolk RP, van Harskamp F, *et al.* Diabetes mellitus and the risk of dementia: the Rotterdam Study. *Neurology* 1999; 53: 1937-1942

6. Luchsinger JA, Tang MX, Stern Y, *et al.* Diabetes mellitus and risk of Alzheimer's disease and dementia with stroke in a multiethnic cohort. *Am J Epidemiol.* 2001; 154: 635-641

7. Peila R, Rodriguez BL, Launer LJ. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: the Honolulu-Asia Aging Study. *Diabetes* 2002; 51: 1256-1262

8. MacKnight C, Rockwood K, Awalt E, *et al.* Diabetes mellitus and the risk of dementia, Alzheimer's disease and vascular cognitive impairment in the