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ORIGINAL ARTICLE

INCREASES IN PLASMA Aβ40 LEVELS AND THE Aβ40/42 RATIO IN PATIENTS WITH DIABETES MELLITUS AND DEMENTIA

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Abstract **Aim:** The plasma Aβ40/42 ratio is a possible biomarker for the onset of Alzheimer's disease (AD). We here measured plasma Aβ40 and Aß42 levels in patients with diabetes mellitus (DM) and dementia in order to clarify the relationship between DM and AD.
Methods: Fifty-three patients, including 33 patients with DM and 25 patients with dementia, were assessed using the Mini-mental state examination (MMSE) and brain MRI, plasma Aβ40 and Aβ42, blood sugar levels, and HbA1c % were measured, and the genotype of apolipoprotein E was determined. Plasma Aβ levels and blood sugar levels were measured in 16 out of 53 patients, following fasting and 2 hours after breakfast.
Results: Plasma Aβ40 levels and the Aβ40/42 ratio were increased in the DM with dementia group ($p<0.01$, $p<0.001$), while HbA1c % correlated with the Aβ40/42 ratio in the non-dementia group. MMSE scores were also associated with the plasma Aβ40/42 ratio and Aβ40 levels in the non-dementia group, independent of the presence of ApoEε4. We did not observed significant direct responses of plasma Aβ protains to an increase in blood sugar levels.
Conclusion: These results suggest that plasma Aβ metabolism are closely related chronic hyperglycemia before the onset of dementia.

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Key words: Dementia; Diabetes mellitus; HbA1c; Plasma Aβ40; Plasma Aβ40/42ratio.

Introduction

Recent studies identified diabetes mellitus (DM) as a risk factor for not only vascular dementia (VaD), but also Alzheimer's disease (AD)[1-3]. The Rotterdam Study and Hisayama Study also reported that DM was a significant risk factor for all-cause dementia and AD[4, 5]. Uncontrolled DM has been shown to increase the risk of AD and VaD[6]. Furthermore, the severity of DM has been associated with cognitive function and a greater decline in the elderly[7]. The Alzheimer's Disease Neuroimaging Initiative (ADNI) study found that subjects with baseline normal blood sugar levels exhibited less of a decline in cognition and whole-brain volume as well as lower conversion rates from MCI to AD[8]. However, 2 major prospective studies showed that glucose intolerance and insulin resistance were not associated with AD pathology[9] and that the rate of cognitive decline was slower in AD patients with DM[10].

The deposition of senile plaque amyloids consisting of aggregated Aβ, the appearance of neurofibrillary tangles, and neuronal cell loss are hallmark characteristics of AD pathology. Two major species of Aβ, Aβ40 and Aβ42, are

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processed from amyloid β precursor protein (APP), with Aβ42 containing more hydrophobic amino acids in the C-terminus than Aβ40. Aβ42 is the early and causal component of amyloid deposits as diffuse plaques in the AD brain, followed by Aβ40 deposits in core plaques and cerebral blood vessels. Recent studies confirmed that cerebrospinal fluid Aβ42 and Aβ40 were the most sensitive biomarkers for predicting and diagnosing AD[11-13]. Plasma Aβ42 and Aβ40 can be assayed and the plasma Aβ40/Aβ42 ratio has recently been suggested as a biomarker of and major risk factor for AD[12-15]. A relationship has also been reported between cognitive function and the plasma Aβ40/Aβ42 ratio[16-19] .

To clarify the role of DM in the onset of dementia in AD, we here measured plasma Aβ40 and Aβ42 levels in 53 patients in our hospital and revealed that long-term hyperglycemia increased the plasma Aβ40/Aβ42 ratio, leading to clinical dementia.

Materials and Methods

Subjects consisted of 53 patients, including 35 females and 18 males, 33 of whom had DM and 25 had dementia. Thirty out of the 33 DM patients had type 2 DM while 3 had type 1 DM. Of the 25 dementia patients, 18 had AD, 5 had AD with cerebrovascular disease, and 2 had dementia with Lewy bodies. Regarding the clinical backgrounds of the 33 patients with DM, the mean duration of the disease was 16.1 years. Fourteen out of 33 patients were treated with insulin therapy. Half of these patients had diabetic microangiopathic complications such as retinopathy or nephropathy.

Neurological examinations, the Mini-mental state examination (MMSE), brain MRI, and a volumetric analysis using the Voxel-based Specific Regional analysis system for AD (VSRAD plus)[20] were carried out. The diagnosis of DM depended on the diagnostic criteria of the Japanese Diabetes Society in 2010. Borderline glucose intolerance cases were excluded from DM patients in order to examine its effects on cognition under chronic hyperglycemia. The diagnosis of dementia was dependent on ICD10 and core clinical criteria by the National Institute on Aging and Alzheimer's disease association workgroups (AAN/AA). Neuropsychiatric examinations using HDS-R (Hasegawa dementia rating scale-revised), clock-drawing, cube copy, kana pick-up test, Kohs block design test, test your memory (TYM) Scale were carried out. Brain MRI (Hitachi APERTO INSPIRE 0.4 tesula) was conducted on all patients. Hippocampal and whole brain atrophy was analyzed by VSRAD plus.[20]

Plasma Aβ40 and Aβ42 levels, the Aβ40/42 ratio, the genotype of apolipoprotein E, blood glucose levels and serum C-peptide immuno-reactivity (CPR), HbA1c %, and body mass index (BMI) were examined after informed consent and the agreement of patients and their families had been obtained. Blood samples were collected casual time in outpatient, and plasma was separated by centrifugation at 3,000 rpm for 10 minutes and stored at −45℃ before the measurement of Aβ40 and Aβ42 using WAKO ELISA kits.[13] Plasma Aβ40 and Aβ42 levels and blood glucose levels and serum CPR were measured in 14 out of 33 DM patients following fasting time and 2 hours after breakfast in hospitalized patient.

Statistical analysis

Statistical analyses were performed using SPSS ver11.01 (SPSS Inc.) and ver 22 and Graph Pad prism 6 (Graphpad Software, Inc.) using the Mann–Whitney U test, a one-way ANOVA, student t test, and paired *t*-test.

Table 1 Basic Patient Characteristics

	Total	Diabetes Mellitus (−)		Diabetes Mellitus (+)	
		Dementia (−) (DM−DEM−)	Dementia (+) (DM−DEM+)	Dementia (−) (DM+DEM−)	Dementia (+) (DM+DEM+)
Number	53	10	10	18	15
Mean age(years)	76.2 ± 9.3	73.3 ± 11.6	82.6 ± 5.4	70.9 ± 8.5	80.2 ± 6.3
Female/male	35/18	9/1	8/2	10/8	8/7
MMSE score	23.5 ± 4.2	27.2 ± 2.0	21.8 ± 1.9	26.2 ± 2.5	19.3 ± 3.8
HSD-R	22.9 ± 4.7	25.9 ± 2.7	20.1 ± 4.1	25.9 ± 3.3	19.1 ± 3.7
BS mmol/L	7.9 ± 3.2	5.9 ± 1.1	5.8 ± 1.4	9.2 ± 3.7	8.9 ± 3.0
HbA1c %	6.8 ± 1.5	5.7 ± 0.3	5.6 ± 0.4	7.5 ± 1.5	7.4 ± 1.6
BMI	22.8 ± 3.4	22.4 ± 4	21 ± 2.5	23.5 ± 4.1	23.5 ± 2
ApoEε4 n[%]	13 (25%)	2 (20%)	2 (20%)	5 (28%)	4 (27%)
Aβ40 fmol/ml	107.7 ± 45.6	83.7 ± 20.3	105.3 ± 33.6	102.7 ± 50.1	131.2 ± 51.5**
Aβ42 fmol/ml	11 ± 3.8	11.0 ± 2.8	11.9 ± 6.4	10.6 ± 3.2	11.2 ± 2.9
Aβ40/42	9.8 ± 2.7	7.7 ± 0.8	9.5 ± 2.4	9.6 ± 2.8	11.7 ± 2.7***
VSRAD plus					
Hippocampus score	1.6 ± 1.1	1.0 ± 0.6	2.2 ± 1.3*	1.1 ± 0.7	2.4 ± 1.1***
Whole Brain %	11.4 ± 4.4	7.8 ± 4.1	13.0 ± 4.1**	9.8 ± 3.4	14.8 ± 2.8***

DM: Diabetes Mellitus; DEM: dementia; MMSE: Mini-mental state examination score; HDS-R: Hasegawa dementia rating scale-revised; BS: casual blood sugar level, mmol/ml; BMI: Body mass index; VSRAD plus: Voxel-based Specific Regional analysis system for Alzheimer's Disease plus version, Hippocampus score: a Z-score in the medial temporal area including the entorhinal cortex, and whole brain % means the percentage of the voxel area showing atrophy (> Z-score 2) relative to the whole brain normal control database.

Results

The basic profiles of our subjects were described in Table 1. In the 20 subjects without DM (DM−), 10 patients did not have dementia (DM−DEM− group) while the other 10 did (DM−DEM+ group). Among the 33 subjects with DM (DM+), 18 did not have dementia (DM+DEM− group) while 15 did (DM+DEM+ group). No significant differences were observed in the mean age with or without DM; however, it was approximately 10 years higher in the groups with dementia. Mean MMSE scores were 27.2 ± 2.0 in the DM−DEM− group, 21.8 ± 1.9 in the DM−DEM+ group, 26.2 ± 2.5 in the DM+DEM− group, and 19.3 ± 3.8 in the DM+DEM+ group. No significant difference was observed in the frequency of ApoEε4 among these groups. Mean casual blood glucose levels were 5.9 ± 1.1mmol/ml in DM−DEM−, 5.8 ± 1.4mmol/ml in DM-DEM+, 9.2 ± 3.7mmol/ml in DM+DEM− and 8.9 ± 3.0mmol/ml in DM+DEM+. High blood glucose levels were observed in the DM (+) groups. HbA1c % were 5.7 ± 0.3 %, 5.6 ± 0.4 %, 7.5 ± 1.5 %, and 7.4 ± 1.6 % in DM−DEM−, DM−DEM+, DM+DEM−, and DM+DEM+, respectively.

Aβ40 levels were 83.7 ± 20.3 fmol/ml in DM−DEM−, 105.3 ± 33.6 fmol/ml in DM−DEM+, 102.7 ± 50.1 fmol/ml in DM+DEM−, and 131.2 ± 51.5 fmol/ml in DM+DEM+. Aβ42 levels were 11.0 ± 2.8 fmol/ml, 11.9 ± 6.4 fmol/ml, 10.6 ± 3.2 fmol/ml, and 11.2 ± 2.9 fmol/ml in DM−DEM−, DM−DEM+, DM+DEM−, and DM+DEM+, respectively. Aβ40/42 ratios were 7.7 ± 0.8, 9.5 ± 2.4, 9.6 ± 2.8, and 11.7 ± 2.7 in DM-DEM-, DM−DEM+, DM+DEM−, and DM+DEM+, respectively. Aβ40 levels were significantly higher in DM+DEM+ ($P<0.01$) than in DM−DEM−, DM−DEM+, and DM+DEM−. However, no significant differences were observed in Aβ42 levels between the groups. The mean Aβ40/42 ratio was also significantly higher in DM+DEM+ ($P<0.001$) (Fig. 1). These results suggested that DM or dementia elevated Aβ40 levels and the Aβ40/42 ratio in plasma.

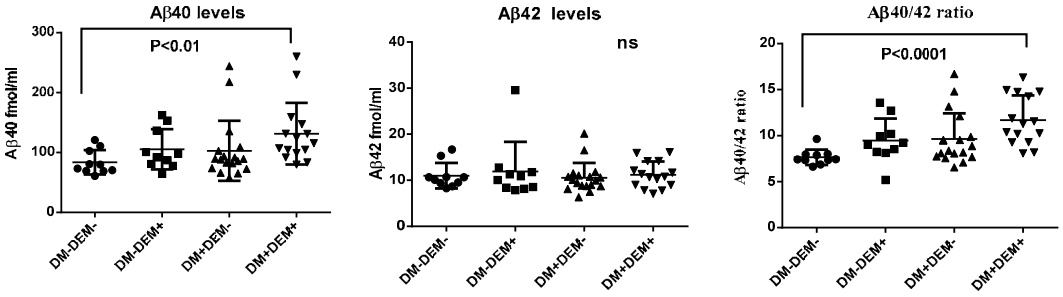

Fig.1 Plasma Aβ40 and Aβ42 levels and the Aβ ratio in four group.
Aβ40 levels and Aβ ratio were significantly higher in the DM+DEM+ group (P<0.01; P <0.0001, respectively).

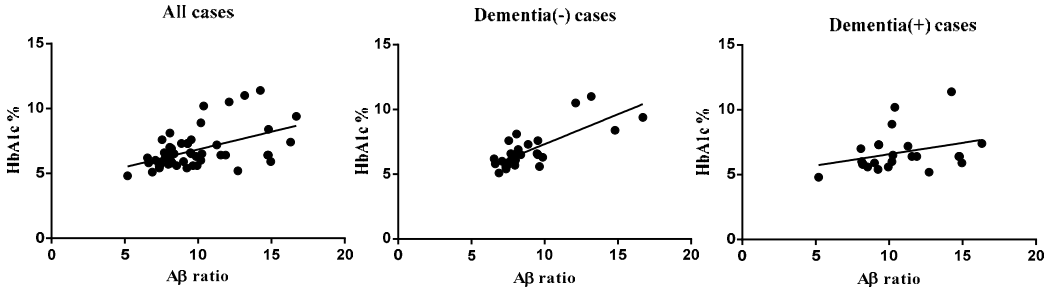

Fig.2 The relationship between the Aβ ratio and HbA1c.
Correlations were observed between HbA1c % and the Aβ40/42 ratio in all subjects (Y=0.2729X + 4.11; P < 0.0002) and in Dementia (−) cases (Y= 0.4613X + 2.709; P < 0.0001).

We analyzed the relationship between HbA1c % and the Aβ40/42 ratio, and found correlations in all subjects (Y=0.2729X + 4.11; P < 0.0002) and the DEM- groups (Y= 0.4613X + 2.709; P < 0.0001) (Fig. 2). To examine this relationship in more detail, we separated all cases into 4 subgroups according to HbA1c % (7 %) and the presence of dementia. The Aβ40/42 ratio was elevated in patients with HbA1c % less than 7 % and with dementia (P <0.001; Fig. 3). Moreover diabetics belong to the group of more than 7 % had high level of the Aβ ratio with or without dementia. These results demonstrated that long-term hyperglycemia road influenced the 40/42 ratio, suggesting that the metabolism of Aβ is altered in DM patients without dementia.

To analyze the relationship between cognitive function and plasma Aβ levels, a linear regression analysis was performed between MMSE scores and the Aβ40/42 ratio and Aβ40 and Aβ42 levels. MMSE scores correlated with the Aβ40/42 ratio (Y = −0.2485X + 14.68; P =0.0045) and Aβ40 levels (Y = −3.482X + 189.8; P = 0.081). However, a relationship was not observed between MMSE scores and Aβ42 levels (Fig. 4). No significant differences were noted in Aβ40 levels (109.8 ± 49.6 SE vs 101.2 ± 31), Aβ42 levels (10.92 ± 2.9 vs 11.56 ± 5.9), or the Aβ40/42 ratio (9.97 ± 2.9 vs 9.36 ± 2.28) between groups without or with ApoEε4 (Fig. 5). These results suggested that the plasma Aβ40/42 ratio and Aβ40 levels were closely related to cognitive function independent of the presence of ApoEε4.

To clarify the direct relationship between blood glucose and plasma Aβ levels, blood samples from 14 inpatients were examined for blood glucose control before breakfast and 2

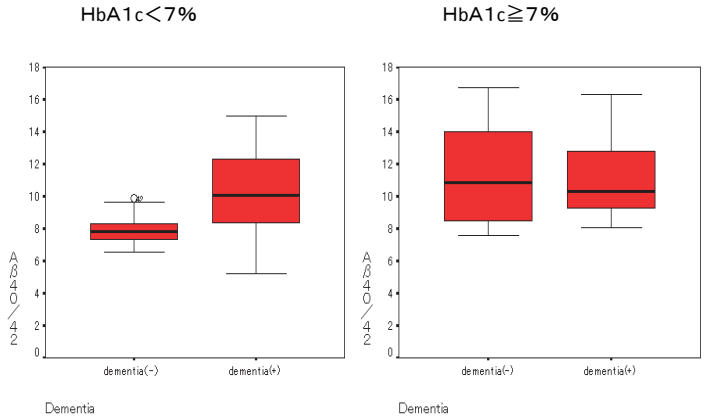

Fig.3 The relation of between the Aβ40/42 ratio and HbA1C in two group according to the blood sugar control.

The Aβ ratio was elevated significantly in patients with HbA1c less than 7% and with dementia (P <0.001), but in patients more than 7% not differed with or without dementia.

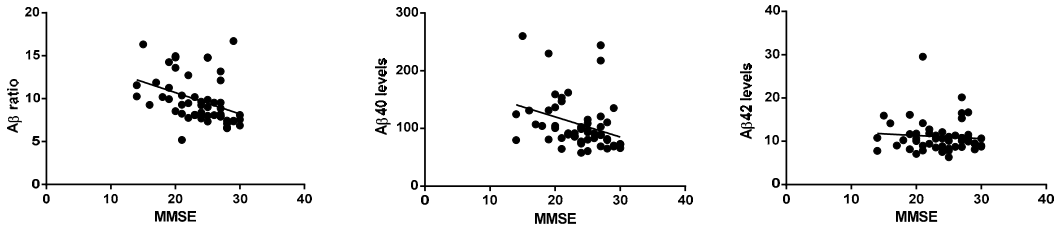

Fig.4 Aβ levels and Cognitive impairment.

MMSE scores correlated with the Aβ ratio (Y = -0.2485X + 14.68; P =0.0045) and Aβ40 levels (Y = -3.482X + 189.8; P = 0.081). However, no relationship was observed between MMSE scores and Aβ42 levels.

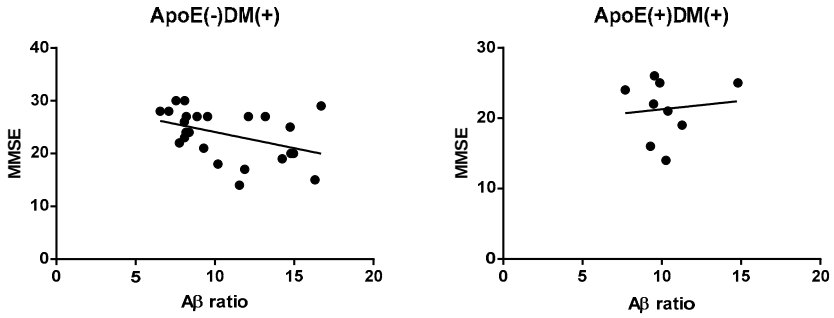

Fig.5 Relationship between the Aβ ratio and ApoEε4 in dementia patients.

A correlation between decreased MMSE scores and a higher Aβ ratio was observed in patients with DM without ApoEε4. However, no significant relationship was found in patients with DM and ApoEε4.

hours after. Figure 6 shows serial alterations in these 4 markers. Blood sugar levels significantly increased from 7.32 mmol/L to 9.50 mmol/L (P<0.002). However, no significant changes were observed in Aβ40 levels (from 148 fmol/ml to 151 fmol/ml), Aβ42 levels (from 11.87 fmol/ml to 12.14 fmol/ml), or the Aβ ratio (from 12.58 to 12.49), suggesting that blood glucose levels did not directly affect plasma Aβ levels or their ratios, daily fluctuations in blood glucose levels

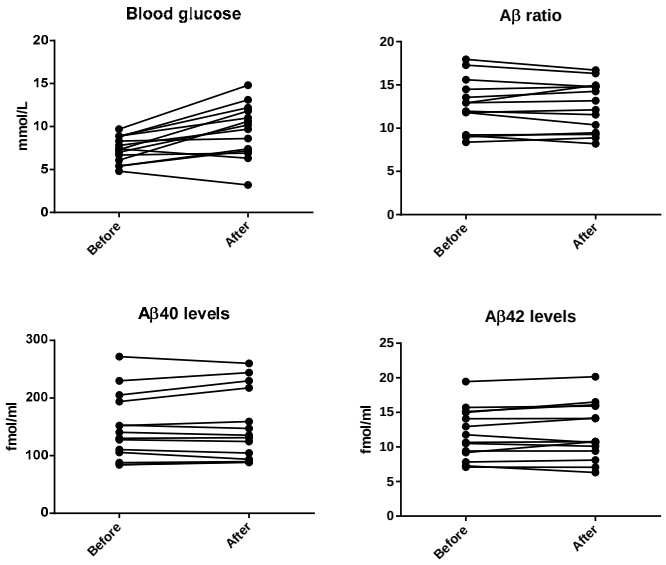

Fig.6 Direct association between blood sugar and Aβ levels.
Blood sugar levels were significantly increased (P <0.002). However, Aβ40 and Aβ42 levels and the Aβ ratio were not significantly affected.

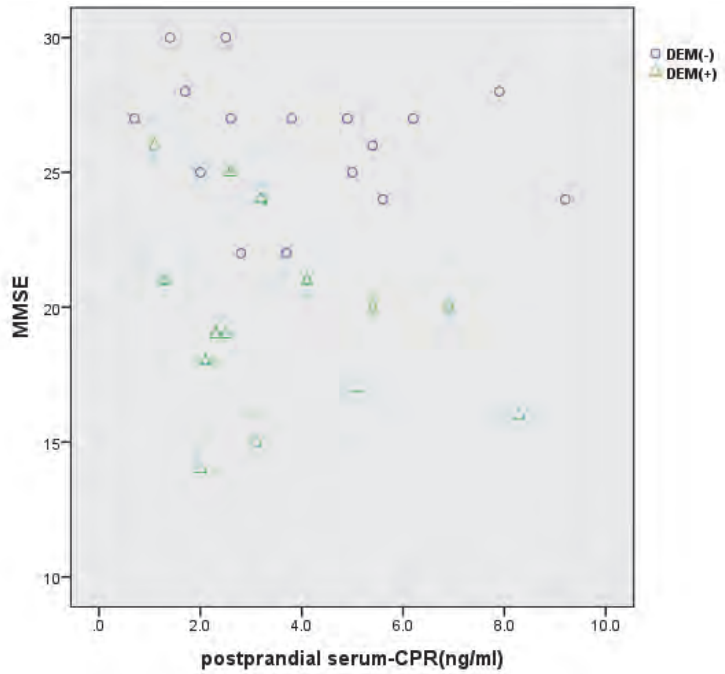

Fig.7 Presence of dementia and the level of postprandial serum CPR in type2 diabetes(n=30).
Patients with dementia showed by open triangles, have any level of serum CPR that indicate endogenous insulin secretion ability.

did not change the Aβ ratio or Aβ40 levels, and long-term disturbances in glucose metabolism affected plasma Aβ metabolism in DM patients. In this series we measured serum CPR that was an index to express endogenous insulin production instead of serum insulin levels because insulin therapy had already done in many patients. The result showed that patients with various insulin production abilities could suffer from cognitive impairment (Fig 7).

Discussion

We herein revealed that plasma Aβ40 levels and the Aβ40/42 ratio were increased in the DM with dementia group and that HbA1c % correlated with the Aβ40/42 ratio, especially in the non-dementia group. A decline in cognitive function was associated with the plasma Aβ ratio and Aβ40 levels in the non-dementia group, independent of the presence of ApoEε4. However, no significant changes were observed in plasma Aβ42 levels in any factors, and we did not detect any direct response of plasma Aβ40 levels or the Aβ40/42 ratio to increased blood sugar levels. These results demonstrated that plasma Aβ40 levels and the Aβ40/42 ratio are closely related with long-term hyperglycemia before the onset of dementia.

A systematic review of 14 eligible longitudinal population-based studies revealed the high risk of VaD and AD in individuals with DM and suggested that vascular disease and alterations in the metabolism of glucose, insulin, and Aβ underlie its pathophysiology.[1] Although the risk of AD is less than that with ApoEε4, DM is a significant independent risk.[2] Hyperinsulinemia disrupts the clearance of Aβ from the brain by competing with insulin-degrading enzymes, and directly affects the accumulation of Aβ in the AD brain.[3, 22] Insulin resistance in the brain also induces central inflammation, cerebrovascular inflammation, oxidative stress, and mitochondrial dysfunction, and advanced glycation end products appear to be important mediators that facilitate the pathology of AD.[23-25]

A prospective cohort study conducted by Graff-Radford *et al*. showed that the plasma Aβ42/40 ratio may be a useful biomarker for predicting the development of mild cognitive impairment (MCI) or AD.[16] The Rotterdam Study demonstrated that a high concentration of Aβ40, but not Aβ42 at baseline was associated with an increased risk of dementia.[17] Low or decreasing plasma Aβ42 levels during a 3- to 6-year follow-up were associated with cognitive decline in MCI and a cognitively intact control cohort.[18] A low plasma Aβ42/40 ratio has also been associated with a greater decline among the elderly without dementia over 8 years.[19] The Australian Imaging Biomarker and Lifestyle (AIBL) study of aging revealed a decrease in the Aβ42/40 ratio in patients with AD that was also inversely correlated with the neocortical amyloid burden. Over 18 months, plasma Aβ42 levels decreased in subjects with MCI and in those transitioning from a healthy condition to MCI.[21] This finding supports our result in which the Aβ40/42 ratio was related to cognitive impairment before the onset of dementia. Our results also indicate that long-term hyperglycemia is a causal factor that increases plasma Aβ40 levels and the Aβ40/42 ratio, leading to cognitive impairment before the onset of dementia.

The relationship between HbA1c % and plasma Aβ levels has not yet been elucidated in detail.[26] Our results clearly show that the Aβ40/42 ratio correlated with HbA1c %. When subjects were limited to patients without dementia, the Aβ40/42 ratio strongly correlated with higher HbA1c % (≥7 %, $p < 0.0001$). These results also suggest that patients with chronic hyperglycemia had impaired Aβ metabolism before the onset of dementia. And another aspect in this study the plasma Aβ40/42 ratio was significantly high in a group with dementia when HbA1c % was less than 7 %. It is thought that the plasma Aβ40/42 ratio is possible biomarker of dementia in without diabetes or in controlled diabetes, but not in poorly controlled diabetes.

In the present analysis of 53 subjects, a negative correlation was observed between plasma Aβ40/42 ratios and MMSE scores. The increase observed in the plasma Aβ40/42

ratio was mainly attributed to elevated Aβ40 levels rather than Aβ42 levels. Van Oijen *et al.* previously showed that increased plasma Aβ40 levels were the most important risk factor for the development of dementia, and that this risk was further increased by a decrease in plasma Aβ42 levels. They also reported that increased Aβ40 levels were often observed in patients with white matter lesions in the brain.[17] Aβ40 has been implicated in vascular toxicity, which may impair the regenerative function of vascular endothelial cells.[29] In addition, the deposition of Aβ40 in the walls of blood vessels has been observed with amyloid angiopathy in the elderly and AD patients. Therefore, the condition of increased Aβ40 levels, such as in hyperglycemia, may facilitate the progression of microangiopathy, leading to cerebral ischemia, which is consistent with the results of the present study.

By a large-scaled study a history of severe hypoglycemic episodes has been associated with a greater risk of dementia, and severely diabetic patients with increased HbA1c % levels are more likely to develop hypoglycemia.[27] An impaired acute insulin response at midlife was associated with an increased risk of AD up to 35 years later.[28] We recognize the fact that dementia could be develop in various insulin secretion ability in type 2 diabetes (Figure7). It seemed dementia in type 2 diabetes have heterogeneity, and the lower insulin secretion group have more frequency of both hypoglycemia and sugar toxic state. On the other hand it is said type 2 diabetes caused dementia from hyperinsulinemia, we suggest the possibility that not severe diabetes with higher insulin secretion ability have more typical pathologies of alzheimer's dementia in comparison with so-called diabetic dementia.

In the present study, no significant differences were observed in the prevalence of ApoEε4 in each group or between those with and without DM, and it reflects that reported in the healthy Japanese population. A negative correlation was noted between MMSE scores and Aβ40/42 ratios in the patient group without ApoEε4, but not in another group with ApoEε4. These results suggest that the plasma Aβ40/42 ratio is related to cognitive impairments independent of ApoEε4 levels.

A previous study reported that Aβ40 levels significantly increased after a glucose load.[25] However, we did not find any significant changes in Aβ40 levels, Aβ42 levels, or the Aβ40/42 ratio after a food load in spite of a significant increase in the mean blood glucose level. Thus, there was no significant short-term change in Aβ levels in response to hyperglycemia after a meal. This may be partly attributed to lower blood sugar levels after a meal than those before receiving anti-diabetic drugs, and also to smaller daily fluctuations in plasma Aβ levels than those in cerebrospinal fluid Aβ levels.

Conclusion

Plasma Aβ40 levels and the Aβ40/42 ratio were increased in DM patients with dementia and HbA1c % correlated with the Aβ40/42 ratio. A cognitive impairment was also related to the plasma Aβ40/42 ratio and Aβ40 levels in DM patients without dementia independent of the presence of ApoEε4. We did not find any direct response of plasma Aβ40 levels or the Aβ40/42 ratio to increased blood sugar levels. These results indicate that plasma Aβ40 levels and the Aβ40/42 ratio are closely related to long-term hyperglycemia before the onset of dementia. Moreover in controlled or not-diabetics the Aβ40/42 ratio may possibly be biomarker of dementia.

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Disclosure statement

The authors declare no conflict of interest.

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