Relationship between olfactory dysfunction and cognitive impairment in elderly patients with type 2 diabetes mellitus

Original Article for Diabetes Research and Clinical Practice

Relationship between olfactory dysfunction and cognitive impairment in elderly patients with type 2 diabetes mellitus

Haruna Sanke MD, [1], Tomoya Mita MD, PhD [1,4], Hidenori Yoshii, MD, PhD[2], Ayako Yokota MD[2], Keiko Yamashiro MD, PhD[2], Noriko Ingaki MD[2], Tomio Onuma MD, PhD[2], Yuki Someya[1], Koji Komiya MD, PhD[1], Yoshifumi Tamura MD, PhD [1,5], Tomoaki Shimizu MD, PhD [1], Chie Ohmura MD, PhD[1], Akio Kanazawa MD, PhD[1,3] Yoshio Fujitani MD, PhD [1], Hirotaka Watada MD, PhD [1,3,4,5]

[1]Department of Metabolism & Endocrinology, [3]Center for Therapeutic Innovations in Diabetes, [4]Center for Molecular Diabetology, [5]Sportology Center, Juntendo University Graduate School of Medicine, Tokyo, Japan, 2-1-1 Hongo, Bunkyoku, Tokyo 113-8421, Japan.

Phone: +81-3-5802-1579. Fax: +81-3-3813-5996

[2] Department of Medicine, Diabetology & Endocrinology Juntendo Tokyo Koto Geriatric Medical Center, Shinsuna 3-3-20, Koto-ku, Tokyo 136-0075, Japan

Phone: +81-3-5802-1579. Fax: +81-3-3813-5996

Short Running title: Olfactory dysfunction and cognitive deficit in elderly diabetics

Word count, text 3,491, abstract 233, number of reference 39, number of table 4

Correspondence: Dr. Tomoya Mita, Department of Metabolism and Endocrinology, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyoku, Tokyo 113-8421, Japan. Phone: +81-3-5802-1579. Fax: +81-3-3813-5996

E-mail: tom-m@juntendo.ac.jp

Abstract

Aims: Recent clinical studies identified the relation between olfactory dysfunction and cognitive impairment in the elderly without type 2 diabetes mellitus. The aim of the present study was to define the relation between olfactory function and cognition in elderly patients with type 2 diabetes mellitus.

Methods: The study participants comprised 250 elderly (age, 68-77, median 72) Japanese outpatient with type 2 diabetes mellitus free of clinically-evident cognitive impairment. Olfactory and cognitive functions were evaluated by the Open Essence (OE) test and Mini-mental State Examination (MMSE), respectively.

Results: Based on the MMSE score, 62.0%, 24.4% and 13.6% of the participants were considered to have no impairment, possible cognitive impairment and probable dementia, respectively. The OE test score of the probable dementia group was significantly lower than other groups. Furthermore, age and serum uric acid were significantly higher in the probable dementia group than other groups. Simple correlation analysis showed positive correlation between the MMSE score and diastolic blood pressure, education, OE test score, total cholesterol, LDL cholesterol, folic acid and negative correlation with age, HbA_{1c}, aspartate aminotransferase, serum adiponectin and urinary albumin excretion. Multivariate regression analysis showed that OE test score correlated significantly and independently with MMSE score (standardized coefficients $\beta = 0.542$, $R^2 = 0.478$, $P<0.01$), in addition to education level, HbA1c and serum adiponectin.

Conclusions: The results suggested the association of olfactory dysfunction with cognitive impairment in elderly patients with type 2 diabetes mellitus.

***Key words*:** Olfactory function, cognitive impairment, type 2 diabetes mellitus, elderly patients

Grant support.

This clinical trial was funded by a grant from the Ministry of Education, Sports and Culture of Japan to CO.

Abbreviations:

AD, Alzheimer's disease;

ANOVA, analysis of variance;

AST, aspartate aminotransferase;

BDI, Beck Depression inventory;

BMI, body mass index;

HDL, high-density lipoprotein-cholesterol;

LDL-C: low-density lipoprotein-cholesterol;

MCI, mild cognitive impairment;

MMSE, Mini-mental State Examination;

OE: Open Essence;

UAE, Urinary albumin excretion

Introduction

One of the main objectives of treatment of type 2 diabetes mellitus is to reduce the onset and development of diabetes-related complications [1]. To achieve these goals, diabetes treatment regimens have been continuously revised. However, self-care management is always an integral part of the overall management of type 2 diabetes mellitus.

Patients with type 2 diabetes mellitus are about 2 to 4 times more likely to develop cognitive impairment and dementia than individuals without type 2 diabetes mellitus [2]. Such impairment can potentially affect diabetes self-care and adherence to medication, with consequent deterioration of glycemic control [3, 4]. In terms of management of type 2 diabetes mellitus, it is crucial to prevent the development of cognitive impairment at an early stage or halt its progression. In this regard, it is important to identify makers for early cognitive impairment in patients with type 2 diabetes mellitus.

Several studies have demonstrated that olfactory dysfunction appears to precede clinical signs of Alzheimer’s disease (AD) or cognitive impairment and may be an early marker of brain changes. Early studies related olfactory dysfunction with neurodegenerative diseases such as AD and Parkinson's disease [5, 6]. Furthermore, olfactory dysfunction was also reported in subjects with mild cognitive impairment (MCI) [7], as well as those with the apolipoprotein E epsilon 4 allele, a strong genetic risk factor for AD [8], and those with family history of AD [9]. Recent clinical studies also identified the relation between olfactory dysfunction and cognitive impairment in the elderly [10, 11]. In addition, patients with olfactory dysfunction were more likely

to make the transition to clinical AD than those without [7]. Taken together, these studies suggest that olfactory function is not only associated with cognitive function, but also could be used as an early marker of future cognitive impairment and AD.

Impaired olfactory function has also been reported in patients with type 2 diabetes mellitus [12]. However, similar to general population, whether olfactory dysfunction is related to cognitive impairment and/or could be an early marker of future cognitive impairment in patients with type 2 diabetes mellitus remains unknown. As a first step, we investigated the relationship between olfactory dysfunction and cognitive impairment in elderly patients with type 2 diabetes mellitus who showed no clinical evidence of cognitive impairment.

Materials and Methods

Subjects. Patients with type 2 diabetes mellitus who met the eligibility criteria described below were invited to participate in the present study through the Diabetes Outpatient Clinic of Juntendo University Hospital (Tokyo, Japan) and Juntendo Tokyo Koto Geriatric Medical Center (Tokyo, Japan). The eligibility criteria were patients with type 2 diabetes mellitus aged more than 65 years with free of clinically-evident cognitive impairment. The exclusion criteria were 1) patients with severe infections within the preceding 2 weeks, 2) patients scheduled for surgery or who had undergone surgery, 3) patients with severe trauma, 4) patients with psychiatric disorders, 5) patients with hypothyroidism, 6) patients with partial or complete olfactory dysfunction associated with sinusitis, allergic rhinitis, and deviated nasal septum, 7) patients with history of brain tumors, 8) patients on steroid treatment, 9) patients with

a MMSE (Mini-mental State Examination; MMSE) score of less than 18 points [they were considered to have severe and moderate cognitive impairment [13]], 10) patients who were considered ineligible based on the assessment of clinical investigators.

Among 312 consecutive elderly patients with type 2 diabetes mellitus who were screened, 256 patients met the above criteria. After being informed of the purpose and procedures of the study, olfaction and cognitive tests were performed between October 2012 and December 2013. Among the 256 patients, 3 with MMSE score of less than 18 points were diagnosed with severe or moderate cognitive deficit for the first time [13] and were excluded from the study. Following blood sampling and laboratory test, 3 patients were found to have hypothyroidism and were also excluded from the study. The remaining 250 patients were the subjects of this cross-sectional study. The study protocol was approved by the Institutional Review Board of Juntendo University Hospital and the study was conducted in accordance with the principles described in the Declaration of Helsinki. All patients provided written informed consent before enrolment in the study. The study has been registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000011073).

MMSE. Cognitive status was evaluated by the MMSE [14].The MMSE is widely used for the assessment of cognitive status in both clinical practice and research [15]. The MMSE includes 10 domain items, which measure orientation to time (5 points), orientation to place (5 points), registration (3 points), attention and calculation (5 points), recall (3 points), naming and repetition (3 points), comprehension (3 points), reading ability (1 point), writing ability (1 point), and design copy (1 point); the latter

being a brief measure of visual construction. MMSE was assessed in a separate room by a trained physician who was blinded to the results of the olfaction test. The MMSE scale ranges from 0 to 30, with a higher score indicating better cognitive performance. Patients were divided into three groups: probable dementia group (18≤MMSE score≤ 23), possible cognitive impairment group (24≤MMSE score≤26), and no impairment group (MMSE score ≥27) [16].

Olfaction test. Smelling sensation was assessed using the Open Essence test (OE test, Wako Pure Chemical Industries). The OE test was developed as an olfactory identification assessment for Japanese population, and has been used in recent studies [17, 18]. It includes a self-completed olfaction test kit, with which the subject identifies various types of odors using specially prepared paper cards. The OE test consists of 9 odorants, including Indian ink, wood, perfume, menthol, orange, curry, cooking gas, rose, hinoki (Japanese cypress wood). In addition, we also used three other odorants; socks that smell of sweat, condensed milk, and roasted garlic, to test olfaction. For each odorant, the subject was presented with a card showing four odor names and two alternative answers (unknown and not detected) and was asked to select the correct answer. A score of 12 points represents correct identification of all types of odorants, while lower scores indicate olfactory dysfunction.

Assessment of depression status. The BDI (Beck Depression inventory)-II is a 21-item questionnaire designed to assess hopelessness, irritability, cognition, guilt, fatigue, weight loss, and sexual interest, in adults and adolescents [19]. Each of the 21

items measures the presence and severity of a somatic or cognitive symptom of depression, rated on a 4-point scale ranging from 0 to 3. The ratings are summed, yielding a total score ranging from 0 to 63. A high score represents depressive states. The BDI-II has been validated as a sensitive, specific, and predictive tool for quantitative assessment of the severity of depression [19].

Other clinical parameters. Body mass index (BMI) was calculated using the formula of weight in kilograms divided by the square of height in meters. Subjects were asked whether they were currently smokers, had smoked, had stopped smoking, the date of stopping smoking, and the number of cigarettes smoked per day. The subjects were classified according to the smoking status into non-smokers, former smokers or current smokers. Former smokers were those who had not smoked during the 6 month receding the study. Estimation of total number of cigarettes (Brinkman Index) was calculated by cigarettes smoked per day times smoking duration (years). Alcohol intake was defined as total daily intake of ethanol, which was estimated in grams according to the average number of grams in a serving of each type of alcoholic beverage. Medications and complications of type 2 diabetes mellitus were also assessed. Patients with simple, preproliferative or proliferative retinopathy were defined as having retinopathy by trained ophthalmologists. Nephropathy was diagnosed in the presence of microalbuminemia (30 mg/g creatinine). Neuropathy was diagnosed in patients with two or more of the following three components: presence of symptoms, absence of ankle tendon reflexes or abnormal scores of vibration perception threshold using a tuning fork. History of cerebrovascular disease was

defined as ischemic or hemorrhagic stroke and carotid endarterectomy. Cardiovascular disease was defined as history of coronary artery disease, including angina, myocardial infarction, percutaneous coronary angioplasty or coronary artery bypass graft surgery. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg, and/or diastolic blood pressure ≥ 90 mm Hg, and/or taking anti-hypertensive medication. Hyperlipidemia was defined by fasting serum total cholesterol ≥ 220 mg/dl, low-density lipoprotein cholesterol (LDL-Cholesterol) ≥ 140 mg/dl and/or high-density lipoprotein cholesterol (HDL-cholesterol) <40 mg/dl, and/or triglyceride ≥150mg/dl, and/or taking lipid lowering medication.

Biochemical tests. Blood samples were obtained after overnight fast. Serum lipids [total cholesterol, HDL-cholesterol, LDL-Cholesterol, triglycerides], HbA1c (National Glycohemoglobin Standardization Program) and glucose, aspartate aminotransferase (AST), alanine aminotransferase, ɤ-glutamyl transpeptidase and uric acid were measured with standard techniques. Highly sensitive C-reactive protein, vitamin B12, folic acid, and adiponectin were outsourced to a private laboratory (SRL Laboratory, Tokyo). Urinary albumin excretion (UAE) was measured by improved bromocresol purple method using a spot urine sample.

Statistical analysis. Results are presented as mean±SD or median (interquartile range: 25% to 75%) for continuous variables or number (proportion) of patients for categorical variables. Data distribution was tested for normality using the Shapiro-Wilk test. Some parameters were logarithmically transformed to approximate

normal distribution. Patients were divided into three groups, the probable dementia group, the possible cognitive impairment group and the no impairment group based on the MMSE score. Multiple comparison test was performed to compare the scores of each variable among the three groups and within a group. Comparisons among groups were tested statistically by the χ^2 test for non-continuous variables and by one-way ANOVA (analysis of variance) for continuous variables, followed by Tukey-Kramer multiple test for normally distributed continuous variables and by the Kruskal-Wallis H test, followed by with Steel-Dwass multiple comparisons post-test for the non-normally distributed variables.

The correlations of potential risk factors for low MMSE score were assessed by Spearman's rank correlation coefficient. Stepwise multiple linear regression analysis was performed to determine those variables that independently and significantly correlated with the MMSE score. Only variables with $P<0.05$ in univariate analyses were included in this model. Especially, the natural log-transformed values of age, education, MMSE score, OE test score, HbA1c, AST, folic acid, adiponectin and UAE were used in multivariable analysis to approximate normal distribution.

Statistical tests were two-sided with 5% significant level. Data were analyzed using the Statistical Package for Social Science computer software program, version 18 (SPSS Inc., Chicago, IL) and the Statistical Analysis System software program, version 10.2 (SAS Institute Inc.) only for Steel- Dwass multiple comparisons post-test.

Results

Table 1 lists the characteristics of the study participants. The median age of the

participants was 72 years and 52.4% of subjects were males. At the time of the study entry, the median duration of diabetes was 14 years with a median HbA_{1c} of 7.1% (54.1(mmol/mol)). The median MMSE score was 27.0 points and the median OE test score was 7.0 points. Blood pressure and serum lipid levels were relatively well controlled. With regard to the treatment of type 2 diabetes mellitus, 167 (66.8%) were on oral hypoglycemic agents; while 53 (21.2%) patients were on insulin. The type 2 diabetes mellitus -related complications included retinopathy in 85 (35.1%) subjects, nephropathy in 118 (48.4%), and peripheral neuropathy in 160 (65.3%). In addition, 42 (20.8%) patients had history of cerebral vascular disease, and 45 (18.3%) subjects had history of cardiovascular disease.

Based on the MMSE score, no impairment of cognitive function was noted in 62.0% of the participants, while possible cognitive impairment was diagnosed in 24.4%, and probable dementia in 13.6% (Table 2). Thus, about 40% of the patients who had not been previously diagnosed with cognitive impairment had low MMSE score. To further clarify the clinical feature of the patients with cognitive impairment, we compared the clinical parameters among the three MMSE score groups. As shown in Table 2, the education levels in both the dementia group and possible cognitive impairment group were significantly lower than the normal group. Age and serum uric acid level were significantly higher in the probable dementia group than the possible cognitive impairment and no impairment groups. UAE and folic acid were significantly higher and lower in the possible cognitive impairment group than the no impairment group, respectively. Significant differences were noted in adiponectin level, diabetic nephropathy, and history of cerebral vascular disease among the three

groups. Intriguingly, the OE test score was significantly lower in the probable dementia group than the possible cognitive impairment and no impairment groups. Furthermore, the OE test score was significantly lower in the possible cognitive impairment group than the normal group.

Finally, we assessed the relation between MMSE score and potential risk factors using Spearman's rank correlation analysis (Table 3). The MMSE score correlated positively with diastolic blood pressure, education level, OE test score, total cholesterol, LDL cholesterol, and folic acid, and negatively with age, HbA1c, AST, serum adiponectin, and UAE. Stepwise multivariate regression analysis that included the above parameters identified OE test score, education level, HbA1c ~~1c~~, and serum adiponectin as independent factors associated with MMSE score (standardized coefficients $\beta = 0.542$, $R^2 = 0.478$, $P<0.01$, Table 4).

Discussion

To our knowledge, this is the first study to report a significant association between olfactory dysfunction and cognitive impairment in elderly patients with type 2 diabetes mellitus.

The results showed a high prevalence (about 40%) of cognitive impairment in elderly patients with type 2 diabetes mellitus who had not been diagnosed with cognitive impairment. This finding was consistent with previous studies demonstrating that 35.0 to 63.3% of patients with type 2 diabetes mellitus had cognitive impairment evaluated by the MMSE test [20, 21]. Taking the high prevalence of cognitive impairment in patients with type 2 diabetes mellitus into

consideration, early detection of cognitive impairment may be required to improve glycemic control, and prevent or delay the development of dementia through proper glycemic control, appropriate drugs and cognitive training.

While the exact mechanism of cognitive impairment in patients with type 2 diabetes mellitus is unknown at present, several mechanisms could be involved under type 2 diabetes mellitus conditions. Hyperglycemia is known to increase the levels of advanced glycation-end products and reactive oxygen species, and the incidence of inflammation, which together could lead to neuronal damage and neurovascular dysfunction [22]. Also, insulin resistance may cause alteration in amyloid metabolism and tau phosphorylation [22]. Furthermore, type 2 diabetes mellitus is a high risk factor for vascular dementia, including both large vessel disease (e.g., stroke) and small vessel disease (e.g., white matters lesion and lacunae). In this regard, cardiovascular risk factors may also lead to cognitive impairment in patients with type 2 diabetes mellitus. Reflecting these findings, recent clinical studies showed that several factors, such as age, gender, glycemic control, duration of type 2 diabetes mellitus, type 2 diabetes mellitus -related complications, hypertension, lipid metabolism, education state, depressive state, physical activity and inflammation are associated with cognitive impairment in patients with type 2 diabetes mellitus [22-26]. In this study, the significant correlations among low MMSE scores and education level, HbA1c, and serum adiponectin level in the multivariate linear regression model are reasonable. Serum adiponectin level was higher in the probable dementia and possible cognitive impairment groups compared with that in the no impairment group. A previous study also demonstrated significant higher serum adiponectin level in

subjects with MCI and AD compared to the control group [27], similar to our finding, although the precise pathomechanism of high adiponectin levels in MCI and AD remain poorly understood.

More importantly, our findings extended the results of previous studies on the general population by demonstrating the graded association between olfactory dysfunction and severity of cognitive function in elderly patients with type 2 diabetes mellitus (Table 2). Furthermore, the OE test score also correlated with the MMSE score even after adjustment for possible risk factors. These findings demonstrate the close relationship between olfactory dysfunction and cognitive impairment in elderly patients with type 2 diabetes mellitus.

Clinical research studies have reported the problem of olfactory dysfunction in patients with type 2 diabetes mellitus [12, 28], although olfactory dysfunction is rarely recognized in clinical practice. Consistent with these findings, we found low OE test scores in the majority of elderly patients with type 2 diabetes mellitus, compared with a score of ≥8 reported in elderly Japanese population [18]. Several hypothetical mechanisms of olfactory dysfunction in patients with type 2 diabetes mellitus have been proposed (29-31). For example, increased oxidative stress was reported to reduce axonal transport rate in the olfactory system of streptozotocin-treated mice, potentially leading to olfactory dysfunction [29]. In addition, the distribution patterns of insulin and insulin receptor are well characterized in the olfactory area in mice [30], and high insulin concentrations were reported in the olfactory bulb, which correlated with reduced olfactory detection in rats [31]. These data suggest the potential roles of hyperglycemia and insulin resistance in the development of olfactory dysfunction. On

the other hand, another possibility is that glycemic control is worsen simply by poor adherence to diabetes medication in subjects with olfactory dysfunction and cognitive impairments. Regarding those issues, a previous clinical report suggested that olfactory dysfunction is associated with old age, male sex and smoking, rather than diabetes-related factors, such as glycemic control and duration of type 2 diabetes mellitus, in patients with type 2 diabetes mellitus [32]. Further studies are required to elucidate the pathogenesis of type 2 diabetes mellitus -related olfactory dysfunction and its relation to cognitive impairment.

Olfactory function includes threshold, discrimination and identification assessed by lowest detectable odorant concentration, ability to differentiate between odorant, and ability to identify odorant, respectively [33]. Recent studies have demonstrated that olfactory identification and discrimination, not olfactory threshold, are closely associated with higher cognitive functions [10, 11, 34]. In the present study, we demonstrated that impaired olfactory identification correlated significantly with cognitive impairment evaluated by MMSE in elderly patients with type 2 diabetes mellitus. Previous studies reported that impaired olfactory identification is a specific predictor of memory decline [35]. Interestingly, studies in patients with mild cognitive impairment demonstrated that impaired olfactory identification and cognitive deficit correlated with volume loss of the hippocampus [36], which is the primary brain region involved in memory formation [37]. Although the exact mechanisms underlying these relationships are largely obscure, AD pathological changes, such as neurofibrillar pathology, were observed in various brain regions of elderly individuals, such as the central olfactory area, entorhinal cortex, and hippocampus

[38]. These changes suggest that neurofibrillar pathology is the culprit pathology underlying both olfactory and memory dysfunctions. In fact, analysis using clinical data in this study setting the OE test score as objective variable identified only age and MMSE as associated factors (data not shown). On the other hand, during an olfactory recognition memory task, functional magnetic resonance imaging studies showed activation of a complex network of brain regions including not only the hippocampus but also prefrontal cortex, lateral and medial parietal areas in humans [39]. Thus, no doubt that olfactory identification may require global cognitive processing including working memory, attention, judgment, language function and decision making, suggesting that impairment of the process of olfactory identification may represent not only memory decline but also general cognitive impairment.

The present study has several limitations. First, the study included a relatively small number of patients and was cross-sectional in design, which did not allow conclusions about cause and effect relationship. In addition, the results do not confirm olfactory dysfunction as a maker of cognitive impairment. Follow-up studies are being conducted at present in our laboratories in order to define the relationship between olfactory dysfunction and subsequent cognitive impairment in the same cohort. Second, we assessed cognitive functions using only the MMSE. While MMSE is considered a screening test for evaluation of cognitive impairment and is the most extensively used, it does not assess all aspects of cognitive function, especially executive function. Thus, other methods need to be used to confirm the diagnosis. Third, we did not examine other aspects of olfactory function apart from olfactory identification. Fourth, the study sample may not be representative of type 2 diabetes

mellitus in Japan, because it was collected from a few hospitals in Tokyo, and thus several potential factors, such as regional differences within Japan should be taken into account. Finally, we did not evaluate the compliance of drugs, which might affect the anthropometric data.

In conclusion, our results point to a close relation between olfactory dysfunction and cognitive impairment in elderly patients with type 2 diabetes mellitus. Further studies are needed to clarify the value of olfactory dysfunction in predicting future cognitive impairment in patients with type 2 diabetes mellitus.

Acknowledgments

We thank all the patients who participated in this study and all the staff at Juntendo University Graduate School of Medicine, Department of Medicine, Metabolism& Endocrinology (Tokyo, Japan), and Juntendo Tokyo Koto Geriatric Medical Center, Department of Medicine, Diabetology & Endocrinology (Tokyo, Japan).

Founding

This clinical trial was funded by a grant from the Ministry of Education, Sports and Culture of Japan to CO.

Contributions

All authors contributed to the study design and were involved in all stages of manuscript processing. HS and TM mainly drafted the manuscript. HS contributed to analysis of research data. All authors were involved in analysis and interpretation of

the data, reviewed/edited the manuscript and approved the final manuscript. HW is the principal guarantor of this work and has full access to all the data in the study and takes responsibility for the integrity of the data and accuracy of data analysis.

Conflict of Interest

TM received research funds from MSD, Takeda and Eli Lilly. TO has received lecture fees from Ono Pharmaceutical Co.. YT has received lecture fees from Takeda Pharmaceutical Co., MSD, Boehringer Ingelheim, Sanofi-Aventis, Eli Lilly, Novartis Pharmaceuticals, Kissei Pharma. AK has received lecture fees from Kissei Pharma, Sanofi and Takeda Pharmaceutical Co. YF has received lecture fees from Novartis Pharmaceuticals and Eli Lilly, research funds from Novartis Pharmaceuticals, MSD and Takeda Pharmaceutical Co. HW has received lecture fees from Boehringer Ingelheim, Sanofi, Ono Pharmaceutical Co., Novo Nordisk Pharma, Novartis Pharmaceuticals, Eli Lilly, Sanwakagaku Kenkyusho, Daiichi Sankyo Inc., Takeda Pharmaceutical Co., MSD, Dainippon Sumitomo Pharm., Kowa Co. and research funds from Boehringer Ingelheim, Pfizer, Mochida Pharmaceutical Co., Sanofi, Novo Nordisk Pharma, Novartis Pharmaceuticals, Sanwakagaku Kenkyusho, Terumo Corp. Eli Lilly, Mitsubishi Tanabe Pharma, Daiichi Sankyo Inc., Takeda Pharmaceutical Co., MSD, Shionogi, Pharma, Dainippon Sumitomo Pharma, Kissei Pharma, and Astrazeneca.

References

[1] Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet. 1998;352:837-53.

[2] Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. Risk of dementia in diabetes mellitus: a systematic review. Lancet neurology. 2006;5:64-74.

[3] Feil DG, Pearman A, Victor T, Harwood D, Weinreb J, Kahle K, et al. The role of cognitive impairment and caregiver support in diabetes management of older outpatients. International journal of psychiatry in medicine. 2009;39:199-214.

[4] Rosen CS, Chow HC, Greenbaum MA, Finney JF, Moos RH, Sheikh JI, et al. How well are clinicians following dementia practice guidelines? Alzheimer disease and associated disorders. 2002;16:15-23.

[5] Serby M, Larson P, Kalkstein D. The nature and course of olfactory deficits in Alzheimer's disease. The American journal of psychiatry. 1991;148:357-60.

[6] Mesholam RI, Moberg PJ, Mahr RN, Doty RL. Olfaction in neurodegenerative disease: a meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases. Archives of neurology. 1998;55:84-90.

[7] Devanand DP, Michaels-Marston KS, Liu X, Pelton GH, Padilla M, Marder K, et al. Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up. The American journal of psychiatry. 2000;157:1399-405.

[8] Murphy C, Bacon AW, Bondi MW, Salmon DP. Apolipoprotein E status is associated with odor identification deficits in nondemented older persons. Annals of the New York Academy of Sciences. 1998;855:744-50.

[9] Schiffman SS, Graham BG, Sattely-Miller EA, Zervakis J, Welsh-Bohmer K. Taste, smell and neuropsychological performance of individuals at familial risk for Alzheimer's disease. Neurobiology of aging. 2002;23:397-404.

[10] Schubert CR, Carmichael LL, Murphy C, Klein BE, Klein R, Cruickshanks KJ. Olfaction and the 5-year incidence of cognitive impairment in an epidemiological study of older adults. Journal of the American Geriatrics Society. 2008;56:1517-21.

[11] Wilson RS, Arnold SE, Tang Y, Bennett DA. Odor identification and decline in different cognitive domains in old age. Neuroepidemiology. 2006;26:61-7.

[12] Le Floch JP, Le Lievre G, Labroue M, Paul M, Peynegre R, Perlemuter L. Smell dysfunction and related factors in diabetic patients. Diabetes care. 1993;16:934-7.

[13] Forsell Y, Fratiglioni L, Grut M, Viitanen M, Winblad B. Clinical staging of dementia in a population survey: comparison of DSM-III-R and the Washington University Clinical Dementia Rating Scale. Acta psychiatrica Scandinavica. 1992;86:49-54.

[14] Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. Journal of psychiatric research. 1975;12:189-98.

[15] Crum RM, Anthony JC, Bassett SS, Folstein MF. Population-based norms for the Mini-Mental State Examination by age and educational level. JAMA : the journal of the American Medical Association. 1993;269:2386-91.

[16] Anstey KJ, Burns RA, Birrell CL, Steel D, Kiely KM, Luszcz MA. Estimates of

probable dementia prevalence from population-based surveys compared with dementia prevalence estimates based on meta-analyses. BMC neurology. 2010;10:62.
[17] Makizako M, Makizako H, Doi T, Uemura K, Tsutsumimoto K, Miyaguchi H, et al. Olfactory identification and cognitive performance in community-dwelling older adults with mild cognitive impairment. Chemical senses. 2014;39:39-46.
[18] Fujio H, Doi K, Hasegawa S, Kobayakawa T, Nibu K. Evaluation of card-type odor identification test for Japanese patients with olfactory disturbance. The Annals of otology, rhinology, and laryngology. 2012;121:413-8.
[19] Beck AT, Steer RA, Brown GK. Manual for the Beck Depression Inventory-II. San Antonio, TX: *Psychological Corporation*. 1996.
[20] Ebady SA, Arami MA, Shafigh MH. Investigation on the relationship between diabetes mellitus type 2 and cognitive impairment. Diabetes research and clinical practice. 2008;82:305-9.
[21] Yamazaki Y, Miwa T, Sakurai H, Hanyu H, Iwamoto T, Odawara M. Clinical backgrounds and morbidity of cognitive impairment in elderly diabetic patients. Endocrine journal. 2011;58:109-15.
[22] Umegaki H, Hayashi T, Nomura H, Yanagawa M, Nonogaki Z, Nakshima H, et al. Cognitive dysfunction: an emerging concept of a new diabetic complication in the elderly. Geriatrics & gerontology international. 2013;13:28-34.
[23] Haan MN, Weldon M. The influence of diabetes, hypertension, and stroke on ethnic differences in physical and cognitive functioning in an ethnically diverse older population. Annals of epidemiology. 1996;6:392-8.
[24] Bruce DG, Davis WA, Casey GP, Starkstein SE, Clarnette RM, Foster JK, et al. Predictors of cognitive impairment and dementia in older people with diabetes. Diabetologia. 2008;51:241-8.
[25] Umegaki H, Iimuro S, Shinozaki T, Araki A, Sakurai T, Iijima K, et al. Risk factors associated with cognitive decline in the elderly with type 2 diabetes: baseline data analysis of the Japanese Elderly Diabetes Intervention Trial. Geriatrics & gerontology international. 2012;12 Suppl 1:103-9.
[26] Marioni RE, Strachan MW, Reynolds RM, Lowe GD, Mitchell RJ, Fowkes FG, et al. Association between raised inflammatory markers and cognitive decline in elderly people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. Diabetes. 2010;59:710-3.
[27] Une K, Takei YA, Tomita N, Asamura T, Ohrui T, Furukawa K, et al. Adiponectin in plasma and cerebrospinal fluid in MCI and Alzheimer's disease. European journal of neurology : the official journal of the European Federation of Neurological Societies. 2011;18:1006-9.
[28] Patterson DS, Turner P, Smart JV. Smell threshold in diabet
es mellitus. Nature. 1966;209:625.
[29] Sharma R, Buras E, Terashima T, Serrano F, Massaad CA, Hu L, et al. Hyperglycemia induces oxidative stress and impairs axonal transport rates in mice. PloS one. 2010;5:e13463.
[30] Marks DR, Tucker K, Cavallin MA, Mast TG, Fadool DA. Awake intranasal insulin delivery modifies protein complexes and alters memory, anxiety, and olfactory behaviors. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2009;29:6734-51.

[31] Aime P, Hegoburu C, Jaillard T, Degletagne C, Garcia S, Messaoudi B, et al. A physiological increase of insulin in the olfactory bulb decreases detection of a learned aversive odor and abolishes food odor-induced sniffing behavior in rats. PloS one. 2012;7:e51227.

[32] Weinstock RS, Wright HN, Smith DU. Olfactory dysfunction in diabetes mellitus. Physiology & behavior. 1993;53:17-21.

[33] Eibenstein A, Fioretti AB, Lena C, Rosati N, Amabile G, Fusetti M. Modern psychophysical tests to assess olfactory function. Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology. 2005;26:147-55.

[34] Sohrabi HR, Bates KA, Rodrigues M, Taddei K, Laws SM, Lautenschlager NT, et al. Olfactory dysfunction is associated with subjective memory complaints in community-dwelling elderly individuals. Journal of Alzheimer's disease : JAD. 2009;17:135-42.

[35] Swan GE, Carmelli D. Impaired olfaction predicts cognitive decline in nondemented older adults. Neuroepidemiology. 2002;21:58-67.

[36] Lojkowska W, Sawicka B, Gugala M, Sienkiewicz-Jarosz H, Bochynska A, Scinska A, et al. Follow-up study of olfactory deficits, cognitive functions, and volume loss of medial temporal lobe structures in patients with mild cognitive impairment. Current Alzheimer research. 2011;8:689-98.

[37] Riedel G, Micheau J. Function of the hippocampus in memory formation: desperately seeking resolution. Progress in neuro-psychopharmacology & biological psychiatry. 2001;25:835-53.

[38] Wilson RS, Arnold SE, Schneider JA, Tang Y, Bennett DA. The relationship between cerebral Alzheimer's disease pathology and odour identification in old age. Journal of neurology, neurosurgery, and psychiatry. 2007;78:30-5.

[39] Crane J, Milner B. What went where? Impaired object-location learning in patients with right hippocampal lesions. Hippocampus. 2005;15:216-31.

Table 1. Patients characteristics (n=250)

Age (years)	72.0 (68-77)
Gender (male)	131 (52.4)
Body mass index (kg/m^2)	24.1(21.9-26.3)
Systolic blood pressure (mmHg)	130.1±15.7
Diastolic blood pressure (mmHg)	70.0±11.6
Estimated duration of diabetes (years)	14.0 (8.0-19.3)
Diabetes complications	
Retinopathy (yes)	85 (35.1)
Nephropathy (yes)	118 (48.4)
Peripheral neuropathy (yes)	160 (65.3)
History of cerebral vascular disease (yes)	42 (20.8)
History of cardiovascular disease(yes)	45 (18.3)
Hypertension (yes)	162(64.8)
Hyperlipidemia (yes)	168 (67.2)
Education (years)	12.0 (9.0-13.3)
Mini-mental State Examination score (points)	27.0 (25.0-29.0)
Open Essence test score (points)	7.0 (4.0-8.0)
Beck Depression inventory –II (points)	8.0 (5.0-13.0)
HbA1c (%)	7.1 (6.6-7.6)
HbA1c (mmol/mol)	54.1 (48.6-59.6)
Fasting blood glucose (mg/dl)	124.0 (108.0-148.0)
Total –cholesterol (mg/dL)	190.0±32.2
HDL-cholesterol (mg/dL)	54.0 (45.0-67.3)
LDL-cholesterol (mg/dL)	110.4±27.9
Triglyceride (mg/dL)	84.5 (64.8-120.0)
aspartate aminotransferase (U/L)	20.0 (17.0-24.3)
alanine aminotransferase (U/L)	16.0 (13.0-21.0)
ɤ-glutamyl transpeptidase (U/L)	22.0 (16.0-34.0)
Uric Acid (mg/dl)	5.2± 1.4
B12 vitamin (pg/ml)	426.0 (302.0-610.0)
Folic acid (ng/ml)	10.4 (7.8-14.6)
Adiponectin (µg/ml)	4.7 (3.0-8.2)
Highly sensitive C-reactive protein (ng/ml)	569.5 (239.8-1425.0)
Urinary albumin excretion (mg/g creatinine)	27.2 (8.3-114.8)
Current smoker(yes)	34(13.6)
Alcohol consumption (g/day)	0.0 (0.0-7.8)
Treatment modality	
Diabetes medication (yes)	220 (88.0)
Oral hypoglycemic agents (yes)	167 (66.8)
Insulin therapy (yes)	53 (21.2)
Anti-hypertensive medication (yes)	130(52.0)
Lipid lowering medication (yes)	120(48.0)
Antiplatelet agents (yes)	76 (30.4)

Data are mean±SD or median (interquartile range: 25% to 75%) for continuous variables or number (proportion) of patients for categorical variables.

5

Table 2. Clinical characteristics of the ~~dementia group, mild cognitive impairment group (MCI) and normal group~~ probable dementia group, possible cognitive impairment group and no impairement group (n=250).

	~~Dementia group~~ probable dementia group (18≤ MMSE ≤23) n=34	~~MCI group~~ possible cognitive impairment group (24≤ MMSE ≤26) n=61	~~Normal group~~ no impairment group (MMSE ≥27) n=155	P value
Age (years)	75.5 (71.5-79.5)*†	72.0 (69.0-77.0)	71.0 (68.0-76.0)	0.001
Gender (male)	22 (64.7)	31 (50.8)	78 (50.3)	0.30
Body mass index (kg/m^2)	24.2 (21.3-27.2)	24.1 (21.5-27.0)	24.1 (22.1-25.9)	0.96
Systolic blood pressure (mmHg)	127.7±21.4	130.6±14.7	130.5±14.7	0.63
Diastolic blood pressure (mmHg)	67.4±13.8	68.1±12.2	71.3±10.7	0.07
Estimated duration of diabetes (years)	15.0 (10.0-24.3)	14.0 (8.0-20.5)	13.0 (7.0-19.0)	0.22
Diabetes complications				
Retinopathy (yes)	15 (44.1)	26 (44.8)	44 (29.3)	0.055
Nephropathy (yes)	17 (51.5)	36 (62.0)	65 (42.4)	0.04
Peripheral neuropathy (yes)	24 (70.6)	43 (71.7)	93 (61.6)	0.30
History of cerebral vascular disease (yes)	13 (52.0)	11 (22.9)	18 (14.0)	<0.001
History of cardiovascular disease (yes)	9 (26.4)	9 (15.0)	27 (17.5)	0.36
Education (years)	9.0 (9.0-12.0) †	9.0 (9.0-12.0) ¶	12.0 (12.0-16.0)	<0.001
Hypertension (yes)	20 (58.8)	44 (72.1)	98 (63.2)	0.34
Hyperlipidemia(yes)	21(61.8)	43 (70.5)	104 (67.1)	0.69
Open Essence test score (points)	3.0 (1.0-4.0)*†	5.0 (3.0-7.0) ¶	8.0 (6.0-9.0)	<0.001
Beck Depression inventory –II (points)	7.0 (5.0-14.3)	10.0 (6.0-16.5)	8.0 (5.0-12.0)	0.08
HbA1c (%)	7.4 (6.8-8.0)	7.1 (6.6-7.7)	7.1 (6.6-7.5)	0.18
HbA1c (mmol/mol)	56.8 (50.8-63.9)	54.1 (48.6-60.7)	54.1 (48.6-58.5)	0.18
Fasting blood glucose (mg/dl)	132.0 (118.5-151.5)	121.0 (106.5-148.5)	124.0 (107.0-147.0)	0.36
Total –cholesterol (mg/dL)	185.9±34.3	185.0±31.8	194.2±31.6	0.10
HDL-cholesterol (mg/dL)	49.0 (42.0-60.5)	52.0 (44.0-64.5)	57.0 (45.0-69.0)	0.051
LDL-cholesterol (mg/dL)	108.8±28.4	105.0±28.2	112.9±27.4	0.17
Triglyceride (mg/dL)	94.0 (60.8-129.7)	87.0 (67.0-132.5)	82.0 (63.0-113.0)	0.30
Aspartate aminotransferase (U/L)	21.5 (17.8-26.3)	22.0 (17.5-26.5)	20.0 (17.0-23.0)	0.10
Alanine aminotransferase (U/L)	15.0 (12.0-21.5)	15.0 (12.0-21.0)	17.0 (14.0-22.0)	0.45
ɤ-glutamyl transpeptidase (U/L)	27.0 (17.8-46.5)	21.0 (17.0-35.0)	21.0 (16.0-30.0)	0.16
Uric Acid (mg/dl)	5.9±1.8 *†	5.2±1.4	5.1±1.2	0.006
Vitamin B12 (pg/ml)	408.0 (275.0-678.3)	426.0 (287.5-695.0)	430.0 (314.8-586.8)	0.77
Folic acid (ng/ml)	10.1 (6.5-13.9)	7.9 (6.6-13.2) ¶	10.8 (8.5-15.0)	0.003
Adiponectin (µg/ml)	6.6 (3.4-10.4)	5.8 (3.3-10.5)	4.4 (2.7-7.3)	0.03
Highly sensitive C-reactive protein ~~(mg/dl)~~ (ng/ml)	666.0 (303.0-1367.5)	572.0 (249.0-1560.0)	532.0 (225.0-1330.0)	0.63
Urinary albumin excretion (mg/g creatinine)	39.2 (16.6-152.7)	46.7 (20.4-339.9) ¶	21.1 (6.7-73.9)	0.002
Current smoker (yes)	6 (17.6)	6 (9.8)	22 (14.2)	0.53
Alcohol consumption (g/day)	0.0 (0.0-3.6)	0.0 (0.0-9.1)	0.0 (0.0-7.8)	0.81
Treatment (n/%)				
Diabetes medication (yes)	33 (97.1)	55 (90.2)	132 (85.2)	0.13
Oral hypoglycemic agents (yes)	24 (70.6)	42 (68.9)	101 (65.2)	0.77

Insulin therapy	9 (26.5)	13 (21.3)	31 (20.0)	0.71
Anti-hypertensive medication (yes)	14 (41.2)	37 (60.7)	79 (51.0)	0.17
Lipid lowering medication (yes)	11 (32.4)	33 (54.1)	76 (49.0)	0.12
Antiplatelet agents (yes)	12 (35.3)	21 (34.4)	43 (27.7)	0.50

Data are mean±SD or median (interquartile range: 25% to 75%) for continuous variables or number (proportion) of patients for categorical variables.

P values are for comparison of the three groups.

*P<0.05, ~~dementia group vs. MCI group.~~ probable dementia group vs. possible cognitive impairment group.

†P<0.05 ~~dementia group vs. normal group.~~ probable dementia group vs. no impairment group.

¶P<0.05 ~~MCI group vs. normal group.~~ possible cognitive impairment group vs. no impairement group.

MMSE; Mini-mental State Examination

Table 3. Correlation between Mini-mental State Examination (MMSE) score and various clinical and laboratory parameters (n =250)

	MMSE score (points)	P value*
Age (years)	-0.024	<0.001
Body mass index (kg/m^2)	0.022	0.726
Systolic blood pressure (mmHg)	-0.024	0.709
Diastolic blood pressure (mmHg)	0.143	0.024
Estimated duration of diabetes (years)	-0.115	0.071
Education (years)	0.422	<0.001
Open Essence test score (points)	0.574	<0.001
Beck Depression inventory –II (points)	-0.067	0.292
HbA1c (% or mmol/mol)	-0.153	0.016
Fasting blood glucose (mg/dl)	-0.123	0.052
Total –cholesterol (mg/dL)	0.140	0.026
HDL-cholesterol (mg/dL)	0.110	0.083
LDL-cholesterol (mg/dL)	0.146	0.021
Triglyceride (mg/dL)	-0.057	0.370
aspartate aminotransferase (U/L)	-0.138	0.029
alanine aminotransferase (U/L)	0.072	0.255
ɤ-glutamyl transpeptidase (U/L)	-0.111	0.079
Uric Acid (mg/dl)	-0.054	0.399
Vitamin B12 (pg/ml)	0.005	0.937
Folic acid (ng/ml)	0.133	0.035
Adiponectin (µg/ml)	-0.186	0.003
Highly sensitive C-reactive protein (ng/ml)	-0.055	0.393
Urinary albumin excretion (mg/g creatinine)	-0.183	0.004
Brinkman Index	-0.063	0.322
Alcohol consumption (g/day)	0.076	0.234

*By Spearman's rank correlation analysis.

Table 4. Results of stepwise multivariate regression analysis for Mini-mental State Examination (MMSE) score (n=250).

Independent variable	Mini-mental State Examination score		
	Standard error	standardized coefficient β	P value
Open Essence test score (points)	0.009	0.542	<0.001
Education (years)	0.021	0.225	<0.001
HbA1c (% or mmol/mol)	0.042	-0.145	0.003
Serum adiponectin (μg/ml)	0.007	-0.105	0.033
R^2		0.478	

Only variables with P<0.05 in univariate analyses were included in the model.