ระดับของนิวโรโทรฟิกแฟคเตอร์จากสมองในซีรั่มกับระดับไขมันในซีรั่มและการรู้คิดของผู้ป่วยโรคอัลไซเมอร์คนไทย

SERUM BRAIN-DERIVED NEUROTROPHIC FACTOR, SERUM LIPIDS AND COGNITION IN THAI PATIENTS WITH ALZHEIMER DISEASE

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บทคัดย่อ

นิวโรโทรฟิกแฟคเตอร์จากสมอง (BDNF) เป็นโมเลกุลโปรตีนที่สำคัญที่ส่งเสริมการเปลี่ยนแปลงรูปร่างของเซลล์ประสาท กระตุ้นการเพิ่มแขนงประสาท และปรับความสามารถในการยืดหยุ่นของสมอง BDNF นี้เกี่ยวข้องกับการเรียนรู้และความจำดังนั้นระดับที่เปลี่ยนแปลงไปจึงอาจจะมีบทบาทสำคัญในการเกิดโรคอัลไซเมอร์ และเนื่องจากไขมันเป็นส่วนประกอบหลักของเยื่อหุ้มเซลล์ประสาทเพราะฉะนั้นระดับไขมันที่ผิดปกติในเลือดจึงอาจกระทบการทำงานของเซลล์ประสาทและอาจสัมพันธ์กับการเกิดโรคอัลไซเมอร์ตามมา งานวิจัยนี้มีจุดมุ่งหมายเพื่อวัดระดับ BDNF ในซีรั่ม ระดับไขมันในซีรั่ม และระดับการรู้คิดของผู้ป่วยโรคอัลไซเมอร์เทียบกับกลุ่มควบคุม และหาความสัมพันธ์ระหว่างระดับ BDNF กับค่าต่าง ๆ ที่ตรวจอีกด้วย การศึกษานี้ทำในกลุ่มควบคุม 30 คน และกลุ่มผู้ป่วยโรคอัลไซเมอร์ 10 คน มีอายุเฉลี่ย 61.20±2.04 ปี และ 79.73±2.17 ปี ตามลำดับ โดยใช้คู่มือ Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V, 2013) เป็นเกณฑ์

ในการวินิจฉัยโรคอัลไซเมอร์ และอาศัยเครื่องมือช่วยประเมินระดับความเสื่อมของสมองฉบับภาษาไทย (Thai Mini-Mental State Examination; TMMSE) การตรวจระดับ BDNF ในซีรั่มใช้น้ำยาสำเร็จรูปโดยหลักการ ELISA การตรวจระดับไขมันในซีรั่มใช้หลักการวัดแบบ Colorimetric และวิธี Homogeneous การประเมินทางสถิติถึงความแตกต่างของค่าตัวแปรระหว่างกลุ่มควบคุมกับกลุ่มผู้ป่วยใช้ Mann-Whitney U test และการประเมินความสัมพันธ์ระหว่างระดับ BDNF กับตัวแปรอื่นใช้ Pearson's test โดยโปรแกรม SPSS (เวอร์ชัน 23.0) ผลการศึกษาจากค่าคะแนน TMMSE เฉลี่ย พบว่ากลุ่มผู้ป่วยโรคอัลไซเมอร์มีความรู้ตัวและความเข้าใจที่แย่กว่ากลุ่มควบคุมอย่างมีนัยสำคัญ ($p<0.001$) ระดับ BDNF ในซีรั่มของกลุ่มผู้ป่วยโรคอัลไซเมอร์มีค่าเฉลี่ย (293.77±44.71 µg/mL) ที่ต่ำกว่าค่าของกลุ่มควบคุม (354.89±26.71 µg/mL) อย่างไม่มีนัยสำคัญทางสถิติ ระดับไขมันในซีรั่มของกลุ่มผู้ป่วยโรคอัลไซเมอร์ไม่แตกต่างจากค่าของกลุ่มควบคุมซึ่งอาจเป็นผลจากการรับประทานยาลดไขมันของผู้ป่วย ไม่พบความสัมพันธ์ระหว่างระดับ BDNF กับระดับไขมันในซีรั่มและระหว่างระดับ BDNF ในซีรั่มกับค่าคะแนน TMMSE โดยสรุปการศึกษานี้ยืนยันประโยชน์ของการใช้เครื่องมือวัดระดับความเสื่อมของสมองฉบับภาษาไทยในการวินิจฉัยผู้ป่วยโรคอัลไซเมอร์ และแนะว่าระดับ BDNF ในซีรั่มของผู้ป่วยโรคอัลไซเมอร์ลดลงแม้จะไม่พบนัยสำคัญทางสถิติ อย่างไรก็ตามยังต้องศึกษาเพิ่มเติมโดยใช้กลุ่มผู้ป่วยจำนวนมากเพื่อยืนยันความเหมาะสมที่จะใช้เป็น Biomarker ในโรคอัลไซเมอร์ต่อไป

คำสำคัญ: โรคอัลไซเมอร์ นิวโรโทรฟิกแฟคเตอร์จากสมองในซีรั่ม ไขมันในซีรั่ม เครื่องมือวัดระดับความเสื่อมของสมองฉบับภาษาไทย การรู้คิด

Abstract

Brain-Derived Neurotrophic Factor (BDNF) is a key protein molecule that promotes neuronal differentiation, stimulates neurite outgrowth, and modulates brain plasticity. BDNF is involved in learning and memory, so changes in its level may play a crucial role in Alzheimer's disease (AD). Since lipids are major structural components of neuronal cell membrane, dyslipidemia may affect neuronal functions that somewhat relate to AD. This study aimed to investigate the levels of serum BDNF protein, serum lipids and the cognitive function of Thai AD patients comparing to the healthy controls, and to search for the corelations between BDNF and other studying parameters. Thirty control and ten AD subjects with mean ages of 61.20±2.04 years, and 79.73±2.17 years, respectively, were participated in the study. The criteria of Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V, 2013) was used for AD diagnosis with Thai Mini-Mental State Examination (TMMSE) as a tool to assess cognitive functions. The levels of serum BDNF were analyzed using ELISA kit, while serum lipids were determined by colorimetric and homogeneous methods. The comparison of parameters between the patients and the control groups were statistically assessed by Mann-Whitney U test, whereas the correlations between serum BDNF levels and other parameters were assessed by Pearson's test using the SPSS software (version 23.0). The results of mean TMMSE scores demonstrated that the AD patients had significantly poorer cognitive function than the control subjects ($p<0.001$). The serum BDNF level in the AD patients (293.77±44.71 µg/mL) was lower than that of the control subjects (354.89±26.71 µg/mL) without statistically significant difference. The serum lipid levels in the AD patient were not significantly different from those in the control group, probably affected by the patients' taking of a lipid-lowering medicine. No significant

correlation was observed between serum BDNF and lipid levels as well as between serum BDNF and TMMSE scores. In summary, our findings confirmed the benefit of TMMSE scores in diagnosis of AD, and suggested a decrease in serum BDNF level in the AD patients although insignificant difference was found. Further study, however, in a large number of patients are required to confirm whether BDNF is suitable as a biomarker for AD.

Keywords: Alzheimer's disease, Serum brain-derived neurotrophic factor (BDNF), Serum lipids, Thai mini mental state examination, Cognitive function

Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, accounting for 50-70% of all cases of worldwide dementia [1-2]. In Thailand, the prevalence of AD in the elderly (over 60 years of age) was 9.98% in 2001 [3]. At present, the number of Thai AD patients is over 700,000 and it is expected to be over 1,110,000 in 2030. Thus, AD becomes one of the major public health problems in Thailand.

Brain-derived neurotrophic factor (BDNF), a member of the classic neurotrophin family of growth factors, promotes and regulates neuronal survival, differentiation, synaptogenesis, neurites outgrowth, and synaptic plasticity. BDNF plays role in learning and memory via the activation of the tyrosine kinase B (TrkB) and p75 low-affinity neurotrophin receptors [4-5]. Decreased signaling of BDNF through TrkB leads to impaired spatial memory [6-8].

In vitro and *in vivo* studies show that BDNF has neuroprotective effects against the cytotoxic effects and learning deficits induced by beta amyloid protein (Aβ) [9-10]. AD subjects showed decreased mRNA and protein levels of BDNF in their serum and brain as compared with healthy elderly controls [11-13]. Decreased levels of BDNF also correlate with the severity of dementia. An explanation is that BDNF may constitute a lack of trophic support with an increase of Aβ accumulation leading to progressive degeneration of AD affected brain [14].

It has been observed that higher expression of BDNF slows down cognitive dysfunction. As the level of BDNF in the blood represents brain BDNF level, it was claimed to use as a promising biomarker for evaluating AD progression [15]. Serum BDNF level was found to increase in both preclinical phase of dementia (mild cognitive impairment), and clinical stages of AD [16]. However, the controversy of BDNF levels in AD patients were found in later publications [17], which may be due to differences in the criteria for patients recruitment, or the severity and stage of the disease.

BDNF not only mediates many aspects of neuronal function, but it also plays a primary role in the central regulation of energy balance and body weight. It is expressed in the liver and visceral organs involved in energy, lipid, and glucose homeostasis. The hepatic BDNF might facilitate the risk of dyslipidemia, insulin resistance and liver disease following high-fat diets consumption [18].

Dyslipidemia is identified by increased triglycerides (TG), total choleserol (TC) and low-density lipoprotein-cholesterol (LDL-C) levels but decreased high-density lipoprotein-cholesterol (HDL-C)

level in serum or plasma [19-21]. Imbalance of these lipids are associated with an increasing risk for development of atherosclerosis, hypertension, diabetes, and dementia in ageing population [22-23].

Ageing, which is usually accompanied with multiple systemic malfunctions of the body, may be associated with abnormal lipid metabolism since the brain was found highly enriched in lipids [24-25] consisting of glycerophospholipids, sphingolipids, and cholesterol in roughly equimolar proportions [26]. Dysregulated lipid metabolism could reflect pathological changes in synaptic function and neuronal membranes, leading to cognitive deterioration in elderly [27].

The brain contains about 20% of the total body cholesterol [28] which is abundant in myelin sheaths. Impairment of cholesterol metabolism by neurons is linked to AD and the ageing process. People with higher baseline total cholesterol in serum during midlife had increased AD risk [29], and impaired late life cognition [30]. Moreover, levels of plasma apolipoproteins, which are closely associated with cholesterol, are altered in mild cognitive impairment [31]. Furthermore, a genetic variant of apolipoprotein E (APOE) is found in late-onset AD [32].

Cholesterol is associated with sphingolipids in membranes to form lipid rafts [33] which play a crucial role in the development and maintenance of neuronal plasticity and functions [34]. The lipid rafts anchor many important transmembrane proteins involved in AD pathogenesis, including β site-APP cleavage enzyme: BACE1 and γ-secretase. Cholesterol enhances the ability of BACE1 and γ-secretase to cleave APP, especially in AD brains [35]. The content of lipid rafts is affected by cholesterol concentration, which consequently affects the morphology of cells and the level of oxidative stress [36-37]. A number of studies reported that disturbance of cholesterol metabolism especially higher levels of brain cholesterol concentration are associated with increased risk of AD [38-39], which indicates that changes in cholesterol homeostasis in the brain may lead to detrimental effects on neuronal cells, resulting in neuronal toxicity and apoptosis to further impairment of cognitive function [40].

The formation of Aβ in the brains of animals can be reduced by inhibiting the expressions of cholesterol synthetic enzymes [41]. An explanation is that BDNF is located in the cholesterol-rich lipid raft domains, and it regulates cholesterol metabolism in lipid rafts on neuronal cell membranes by stimulating cholesterol synthesis [42]. Cholesterol overload induced the reduction in BDNF signaling is mediated through alterations in expressions of BDNF and its receptors. However, the variations of serum BDNF and lipids on ageing and cognitive function in AD patients are required to elucidate.

Objectives

This study aimed to investigate the levels of serum BDNF, serum lipids and cognitive function in Thai patients with Alzheimer's disease comparing to those in the control group. Additional objective was to search for the corelations between serum BDNF and TG, BDNF and TC, BDNF and HDL-C, BDNF and LDL-C, as well as BDNF and TMMSE scores of all participants.

Methods

Participants

The present study was approved by the Institutional Human Ethic Committee of Srinakharinwirot University (SWUEC-025/58E). This study was performed during November 2015 to November 2016 in which forty volunteer subjects aged from 45 to 91 years old were informed about the procedure of the study before making their decision to sign written consent form and participate. The participants were divided into 2 groups: control and Alzheimer's disease (AD) groups. The control group comprised 30 healthy subjects (27 females and 3 males). They were recruited from members of the Ageing Club of Srinakharinwirot University, Bangkok, Thailand. Inclusion criteria for recruitment of the control subjects were healthy elderly with no history of diabetic mellitus, hypertension, and dyslipidemia as well as neurological and psychiatic diseases.

The AD groups were composed of ten patients (7 females and 3 males) diagnosed with AD and dyslipidemia. Only those without psychiatic problems such as anxiety disorders and depression were recruited for this study. The patients were regularly received treatment at the Out-Patient Department of Neurology, Her Royal Highness Princess Mahajakri Sirindhorn Medical Center (MSMC), Ongkharak, Nakhon-Nayok, Thailand. The diagnosis of AD was taken by the neurologists at MSMC according to the criteria of Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V, 2013) and the American Psychiatric Association (APA, 2013).

Serum Brain-Derived Neurotrophic Factor Assay

Ten-milliliter blood of the control and AD subjects was collected via left antecubital vein using 21G needle. The blood samples were placed at room temperature until clotted, and then, they were centrifuged at 1,000 rpm for 10 minutes. Serum was separated and kept at -80^{0}C until analysis. Serum BDNF protein was measured using Enzyme Linked Immunosorbent Assay (ELISA) (Milliplex assay kit, Catalogue No. HNDG3MAG-36K, Merck Millipore, Germany) running on Luminex®, Model MAGPIX, Texas, USA. Mouse monoclonal antibodies generated against human BDNF coated onto 96-well immune assay plate were used to capture BDNF from the control and AD serum samples. The biotinylated mouse anti-human BDNF antibodies were used to detect the captured BDNF. After adding streptavidin-horseradish peroxidase conjugate, substrate was added to the plates as color indicator. Finally, a stop solution was added to stop the reaction. Samples were assayed in triplicate. The amount of BDNF protein was determined by measured absorbance at wavelength 450 nm compared with the standard curve.

Serum Lipids Assay

A lipid profile consists of triglycerides, total cholesterol, HDL-C and LDL-C. Serum triglycerides and total cholesterol were assayed by colorimetric method. Both HDL-C and LDL-C were measured by homogenous method. All lipid profile determinations were analyzed on Dimension RXL chemistry analyzer (Dade Behring, USA).

Cognitive Function Determination

Mini Mental State Examination [43] which was modified into Thai Mini Mental State Examination (TMMSE) version was used to assess the subject cognitive function in both the control and AD patient

groups, once throughout this study. The TMMSE is a screening test of cognitive function including orientation, attention, memory, language and visual-spatial skills. The subjects in the control group were classified from the TMMSE scores of higher than 23, and those who had scores lower than 23 were excluded. TMMSE scores of equal to or less than 23 were used as the cutoff-value for AD group. In this study, the AD patients were not categorized into different degrees of severity (mild, moderate and severe stages).

Statistical Analysis

The statistical analysis of differences between the control and AD groups was performed using Mann-Whitney U test. Correlations between BDNF and TG, BDNF and TC, BDNF and HDL-C, BDNF and LDL-C, as well as BDNF and TMMSE scores of all participants were determined by Pearson's test. All statistical analyses were carried out using SPSS software (version 23.0, SPSS Incorporated).

Results

Table 1 shows age, sex, body weight, height, body mass index, vital signs, and Thai Mini Mental State Examination (TMMSE) scores of the control elderly and the AD patients. It demonstrates that the mean age of the control group (61.20 ± 04.2) was significantly lower than that of the AD patient group (79.73 ± 2.17) with the p value of < 0.05. In addition, the mean TMMSE score of the control group (27.62 ± 0.36 points) was significantly higher than that of the AD group (13.82 ± 2.17 points) with the p value of < 0.001 (Table 1 and Figure 1).

Table 1. Physical characteristics: Comparison between the control and the Alzheimer's disease (AD) patient groups.

Characteristics	Control (N=30)	AD Patients (N=10)
Age (year)	61.20±2.04	79.73±2.17 *
Male (N, %)	3 (10%)	3 (30%)
Female (N, %)	27 (90%)	7 (70%)
Body weight (kg)	59.80±1.70	53.97±3.20
Height (cm)	156.23±0.84	158.50±3.08
Body Mass Index (kg/m^2)	17.85±0.99	19.08±0.51
Resting Blood Pressure (mmHg)		
Systolic Blood Pressure	126.94±3.02	128.73±2.01
Diastolic Blood Pressure	78.29±1.56	74.00±3.26
Resting Heart Rate (beat/min, bpm)	74.64±1.73	77.73±2.83
Resting Body Temperature (^{0}C)	36.20±0.10	36.13±0.27
TMMSE scores,	27.62±0.36	13.82±2.17 +

Data show as mean ± S.E.M.; N = number of subjects

* Significant difference of mean age between the control and AD groups ($p < 0.05$).

+ Significant difference of TMMSE scores between the control and AD groups ($p < 0.001$).

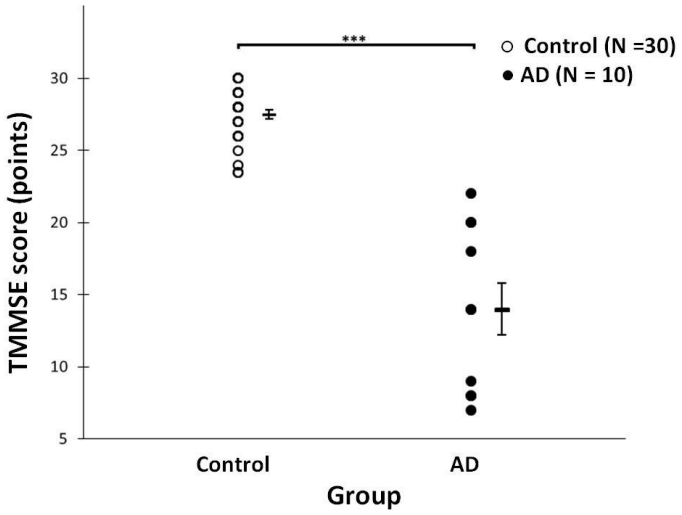

Figure 1. Scattergram showing the individual TMMSE scores in the control (N = 30) and Alzheimer's disease (AD) groups (N = 10); Means (horizontal bars); Standard error (vertical bars); ***significant difference ($p < 0.001$).

Lipid profile consisting of serum levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were analyzed in the control and the AD groups as shown in Table 2. It was found that the mean serum TG, TC, LDL-C and HDL-C concentrations in the control group were 112.83 ± 73.05 mg/dL, 193.78 ± 55.60 mg/dL, 113.65 ± 46.11 mg/dL and 51.02 ± 11.25 mg/dL, respectively whereas those of the AD groups were 109.88 ± 39.35 mg/dL, 193.50 ± 29.49 mg/dL, 115.20 ± 23.79 mg/dL and 49.05 ± 11.34 mg/dL, respectively. Hence, there was no significant difference of all types of lipids between the control and the AD groups in this study ($p > 0.05$).

Figure 2 shows the levels of individual serum BDNF in the control as compared to those of the patients with AD. It demonstrates that the average concentration of BDNF in the AD patients (293.77 ± 44.71 µg/mL) was apparently lower than that of the control group (354.89 ± 26.71 µg/mL). However, no significant difference of BDNF between the two groups was found ($p > 0.05$).

Table 2. Lipid profiles: Comparison between the control and the Alzheimer's disease (AD) patient groups.

Lipids	Control (N=30)	AD Patients (N=10)
HDL (mg/dL)	51.02±11.25	49.04± 11.33
LDL (mg/dL)	113.64±46.10	115.19± 23.79
Total cholesterol (mg/dL)	193.78±55.60	193.50±29.49
Triglycerides (mg/dL)	112.83±73.04	109.88±39.35

Data show as mean ± S.E.M.; N = number of subjects.

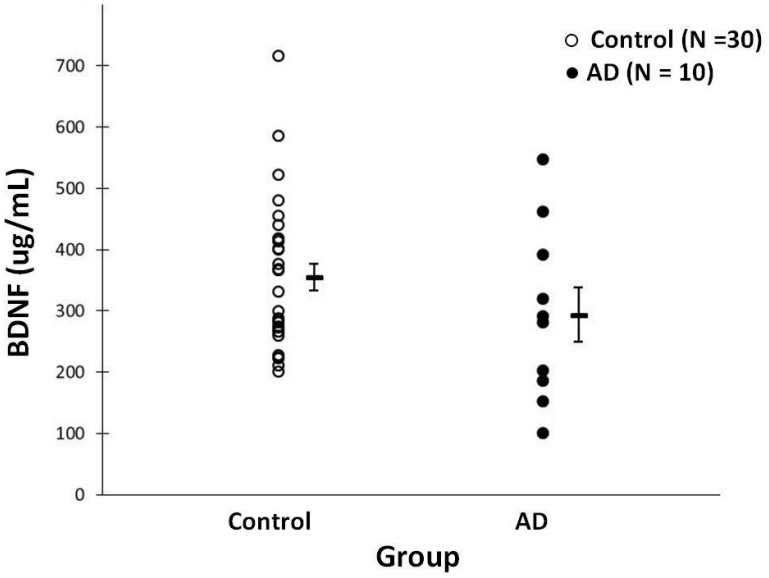

Figure 2. Scattergram showing the individual serum Brain-Derived Neurotrophic Factor (BDNF) concentrations of the control (N = 30) and Alzheimer's disease (AD) patient groups (N = 10); Means (horizontal bars); Standard error (vertical bars).

Correlations between serum BDNF protein levels and lipid profiles in the healthy elderly and the AD patient groups were determined in this study. No significant correlations were observed between serum BDNF and HDL-C levels ($r = 0.072$, $p = 0.658$), serum BDNF level and LDL-C ($r = -0.098$, $p = 0.547$), serum BDNF level and TC ($r = -0.052$, $p = 0.748$), as well as serum BDNF level and TG ($r = 0.128$, $p = 0.433$) (Table 3).

Additionally, the correlation between serum BDNF protein levels and cognitive function represented memory impairments, as measured by TMMSE scores was investigated. Apparently, no significant correlation was observed between serum BDNF protein levels and the TMMSE scores ($r = 0.252$, $p = 0.116$) (Table 3) even though the TMMSE scores in the AD group were significantly lower than those

of the control group (Figure 1). However, at least 4 in 10 of the AD patients in our study had both low TMMSE scores (< 23 points) and low serum BDNF protein levels (< 200 μg/ml).

Table 3. Correlation coefficient (*r*) and *p*-values of the plots between levels of serum Brain-Derived Neurotrophic Factor (BDNF) versus levels of various types of serum lipids, and TMMSE scores in all subjects (N=40). (Number of the control = 30; Number of the AD patients = 10).

Plots	***r***	***p*-value**
BDNF vs. HDL-cholesterol	0.072	0.658
BDNF vs. LDL-cholesterol	-0.098	0.547
BDNF vs. Total cholesterol	-0.052	0.748
BDNF vs. Triglycerides	0.128	0.433
BDNF vs. TMMSE scores	0.252	0.116

HDL = High Density Lipoprotein; LDL = Low-density lipoprotein; TMMSE = Thai Mini Mental State Examination.

Conclusions and Discussion

Our findings, to some extent, support the role of BDNF in neurodegenerative mechanisms as observed in AD Thai patients. We found that the mean age of the AD patients (79.73 ± 2.17) was significantly higher than that of the control elderly (61.20 ± 04.2) while the mean level of serum BDNF in the AD patients (293.77 ± 44.71 μg/mL) was apparently lower than that of the control group (354.89 ± 26.71 μg/mL). Despite statistical insignificance, these might somewhat indicate a negative correlation between serum BDNF level and age, as previously observed by Ziegenhorn, et al. [44]. The observed BDNF level that was higher in our control group might be the result of the higher mean age of AD patients as compared to that of the control group. Besides less number of AD patients, there were limitations to recruit the age-matched healthy controls, and avoidance of patients taking lipid-lowering drugs.

The insignificant difference of BDNF levels between Thai AD patients and the healthy groups in this study may be explained by the degree of severity of AD that there is late compensatory repairment mechanism for neuronal cells recovery and survive [45]. Previous study also reported that serum BDNF protein in the controls was higher than those with mild cognitive impairment, AD or dementia [45]. This circumstance seems to be supported by our results. However, in this study the serum BDNF levels in both controls and AD patients were quite higher (10^4 times) than those in other studies [46-47]. The higher baseline levels of serum BDNF were possibly a result of the differences in patient recruitment criteria based on the fifth edition of DSM:2013, the limited number of AD patients, and the different ELISA commercial assay kit used for determination of serum BDNF level [48]. This occurrence still cannot

be fully explained by a new molecular mechanism, but differences in ethnic, lifestyle behavior, baseline health status of subjects and the severity of disease may be the causes.

Since the disturbance of brain cholesterol metabolism may be associated with some of the pathological features of AD [20], the cholesterol concentrations were expected to be higher in the AD patients than in the healthy controls. However, the AD patients with an underlying dyslipidemia that were enrolled in this study had serum cholesterol level as well as other lipid profile data in the reference ranges. These were probably resulted from regular taking of a lipid-lowering agent (statin) by the AD patients before, during and after enrollment into this study. The recruitment of AD patients with dyslipidemia who have never taken lipid-lowering drug is demanding since it was a confounding factor in interpreting the results. A study in an animal model of AD reported that a cholesterol-lowering drug could also increase BDNF level and improve memory and learning [49].

This study simply diagnosed the AD patients based on TMMSE scores without grading into mild, moderate and severe stages of diseases because of the limited number of Thai AD patients. Previous studies, however, distinguished between the control and the AD patients based on the MMSE scores with grading of AD patients into different degrees of severity [9, 46]. We might be able to more accurately observe the relationship between serum BDNF levels and TMMSE scores if we did categorize the patients more granularly based on their TMMSE scores. However, we found that 4 out of 10 AD patients had both low TMMSE scores (< 23 points) and low serum BDNF protein levels (< 200 μg/ml), which is similar to a previous report by Ventriglia and colleagues [50].

This finding confirmed that serum BDNF protein in the AD patients was lower than that of the controls. However, it is too early to claim that BDNF might be a promising biomarker for diagnosing and monitoring of AD due to small sample size. For further study, we plan to collect data from a larger pool of both elderly individuals and the AD patients. This may help us confirm whether serum BDNF should be a biomarker for future use in AD patients.

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