รายงานวิจัยฉบับสมบูรณ์

ตัวพยากรณ์ที่เป็นไปได้ต่อการเกิดภาวะความจำบกพร่องแบบต่ำ ๆ ในผู้ป่วยโรคเบาหวานชนิดที่ 2

The possible predictors for mild cognitive impairment in patients with type 2 diabetes mellitus

โดย

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Noppamas Sripetchwandee

บทคัดย่อ

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ชื่อโครงการ: ตัวพยากรณ์ที่เป็นไปได้ต่อการเกิดภาวะความจำบกพร่องแบบต่ำๆ ในผู้ป่วยโรคเบาหวานชนิดที่ 2

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บทคัดย่อ:
วัตถุประสงค์/สมมุติฐาน: เป็นที่ทราบกันดีว่า โรคเบาหวานชนิดที่ **2** มีสัมพันธ์กับการเกิดโรคจำเสื่อม โดยเฉพาะอย่างยิ่งภาวะความจำเสื่อมชนิดต่ำ ๆ อย่างไรก็ตาม ตัวชี้วัดการดำเนินไปของภาวะความจำเสื่อมชนิดต่ำ ๆ ในผู้ป่วยกลุ่มนี้ยังไม่เคยมีการศึกษามาก่อน เมื่อไม่นานมานี้ อัตราส่วนระหว่างเม็ดเลือดขาวชนิดนิวโตรฟิลต่อเม็ดเลือดขาวชนิดลิมโฟไซต์ ซึ่งเป็นตัวชี้วัดของภาวะการอักเสบในกระแสเลือดถูกนำมาใช้เป็นตัวพยากรณ์ของโรคมะเร็งและโรคหลอดเลือดหัวใจ แต่อย่างไรก็ตามความสัมพันธ์ระหว่างตัวชี้วัดชนิดนี้กับภาวะความจำเสื่อมชนิดต่ำ ๆ ในผู้ป่วยโรคเบาหวาน ยังไม่เป็นที่ทราบแน่ชัด
วิธีการทดลอง**:** อาสาสมัครที่สุขภาพดี จำนวน 30 ราย และผู้ป่วยโรคเบาหวานชนิดที่ 2 จำนวน 215 ราย ที่มีอายุระหว่าง 45-65 ปี จากโรงพยาบาลสารภี อำเภอสารภี และโรงพยาบาลมหาราชนครเชียงใหม่ อำเภอเมือง จังหวัดเชียงใหม่ จะถูกรวบรวมเพื่อเข้าร่วมการวิจัย โดยข้อมูลทั่วไป ได้แก่ อายุ, ระดับการศึกษา, ประวัติโรคต่าง ๆ, ยาที่ใช้อยู่ในปัจจุบัน และ ข้อมูลทางร่างกาย ต่าง ๆ ได้แก่ น้ำหนัก, ส่วนสูง, รอบเอวและรอบสะโพก, ความดันโลหิตและอัตราการเต้นของหัวใจ จะถูกเก็บข้อมูล นอกจากนี้อาสาสมัครจะได้รับการเจาะเลือดเพื่อวัดระดับ metabolic profile ต่าง ๆ เช่น Fasting plasma insulin, fasting plasma glucose, HbA_{1C}, lipid profiles (total cholesterol, triglyceride, HDL, LDL), serum soluble CD99 (sCD99), ไลโปโพลิแซคคาไรด์ในเลือด และตรวจความสมบูรณ์ของเลือด (complete blood count) ค่าอัตราส่วนระหว่างเม็ดเลือดขาวชนิดนิวโตรฟิลต่อเม็ดเลือดขาวชนิดลิมโฟไซต์ คำนวณ

ได้จากผลเลือด นอกจากนี้ อาสาสมัครทุกรายจะถูกทำการทดสอบ Montreal Cognitive Assessment (MoCA) test เพื่อประเมินภาวะความจำเสื่อมชนิดต่ำ ๆ

ผลการทดลอง: จากผลการทดลองพบว่า อุบัติการณ์การเกิดภาวะความจำเสื่อมชนิดต่ำ ๆ (คะแนน MoCA ต่ำกว่า 26) ในผู้ป่วยโรคเบาหวาน สูงถึง 93% นอกจากนี้ยังพบว่า อายุ, ดัชนีมวลกาย, ระดับน้ำตาลในเลือดขณะอดอาหาร และ อัตราส่วนระหว่างเม็ดเลือดขาวชนิดนิวโตรฟิลต่อเม็ดเลือดขาวชนิดลิมโฟไซต์ มีความสัมพันธ์อย่างมีนัยสำคัญต่อคะแนน MoCA ในผู้ป่วยกลุ่มนี้ อย่างไรก็ตามมีเพียง ดัชนีมวลกายและอัตราส่วนระหว่างเม็ดเลือดขาวชนิดนิวโตรฟิลต่อเม็ดเลือดขาวชนิดลิมโฟไซต์ที่เป็นปัจจัยอิสระมีความสัมพันธ์อย่างมีนัยสำคัญต่อคะแนน MoCA score

สรุปผลการทดลอง: ภาวะความจำเสื่อมชนิดต่ำ ๆ พบได้ทั่วไปในผู้ป่วยโรคเบาหวานชนิดที่ **2** ทั้งนี้ อัตราส่วนระหว่างเม็ดเลือดขาวชนิดนิวโตรฟิลต่อเม็ดเลือดขาวชนิดลิมโฟไซต์ อาจจะใช้ทำนายประสิทธิภาพของความสามารถในการจำที่แย่ลงในผู้ป่วยกลุ่มนี้

คำหลัก: : ภาวะความจำเสื่อมแบบต่ำ ๆ**;** โรคเบาหวานชนิดที่ **2;** อัตราส่วนของนิวโตรฟิลต่อลิมโฟไซต์**;** โรคอ้วน

Abstract

Project Code: TRG5980200

Project Title: The possible predictors for mild cognitive impairment in patients with type 2 diabetes mellitus

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Project Period: 24 months

Abstract:

Background: Type 2 diabetes mellitus (T2DM) has been known to be associated with cognitive impairment particularly in the mild cognitive impairment (MCI). However, a prognostic biomarker for the MCI condition in these patients has not been investigated. Recently, the neutrophil-lymphocyte ratio (NLR), an indicator of systemic inflammation, has been used as a predicted biomarker for the development of several types of cancers and cardiovascular diseases. Nevertheless, its association with the MCI condition in T2DM patients has not known.

Aim of the study: The present study aimed to investigate the correlation between NLR and cognitive function in these patients.

Methods: A thirty control subjects and a total of 215 T2DM patients were enrolled in the present study. Demographic data including age, level of education, history of sickness and medication, physical examination (body weight, height, waist and hip circumference, blood pressure and heart rate) were collected. In addition, metabolic parameters including fasting plasma insulin, fasting plasma glucose, HbA_{1C}, plasma lipid profiles (total cholesterol, triglyceride, HDL, LDL), serum soluble CD99 (sCD99), serum lipopolysaccharide, and the complete blood count were determined. NLR level was calculated by the ratio of neutrophils to lymphocytes derived from

blood measurement. Moreover, all individuals were performed the Montreal Cognitive Assessment **(**MoCA**)** test to determine the MCI condition.

Results: From the results, a 93% prevalence of MCI, (MoCA score of less than 26), was found diabetic patients. A significant correlation between age, body mass index, fasting plasma glucose, and NLR and the individual MoCA score was shown in these patients. However, BMI NLR were the strongest independent factor significantly correlated with MoCA score.

Conclusion: MCI is common in T2DM patients. Obesity and NLR levels may predict poorer cognitive performance outcomes in these patients.

Keywords: mild cognitive impairment; type 2 diabetes mellitus; neutrophil-lymphocyte ratio; obesity.

เนื้อหางานวิจัย (Executive summary)

บทนำ (Introduction)

Type 2 Diabetes mellitus (T2DM) has been characterized by hyperglycemia in association with insulin resistant condition [2]. The etiology of T2DM could be from both environmental factors and genetic factors [3]. Individual will be diagnosed as T2DM condition when their plasma glucose level reached at the diagnostic criteria, according to recent study (1). The diabetic complications can be categorized into 2 groups including microvascular complications (e.g., nephropathy, retinopathy and neuropathy) and macrovascular complications (e.g., peripheral vascular disease, cerebrovascular disease and cardiovascular disease) (2). However, good control in plasma glucose levels has been demonstrated to delay those complications (3, 4).

In addition, several studies have revealed the development of cognitive impairment in T2DM patients (5-9). Recent clinical studies revealed that the high glucose level from both diabetic and non-diabetic subjects such as is correlated with the risk for cognitive impairment or dementia (10, 11). Furthermore, various complications under diabetes of vascular disturbances such as a damage in nervous structure; blood brain barrier and metabolic disorders including insulin resistance, glucose toxicity and the formation of advanced glucose end-products (AGEs) could be responsible for the development of brain dysfunction. Those findings suggested T2DM could further contribute to the development of cognitive impairment.

Mild cognitive impairment or MCI has been described as a state of cognitive impairment in which the degeneration in cognitive function is more than normal level [21]. Patients with MCI has been characterized by the cognitive impairment, but the severity of cognitive decline was not

sufficient to develop dementia [22, 23]. The clinical feature of MCI is a high risk to the development of Alzheimer's disease (AD) [24, 25]. Moreover, previous studies have demonstrated the positive correlation of diabetes mellitus and MCI (12-14). Several factors in T2DM patients, including the duration of diabetes, use of glucose-lowering drugs, degree of glycemic control can be the predictors for MCI in those patients (15). Although several factors in T2DM have been reported to be associated with the cognitive decline, the prognostic markers for the early detection of MCI in T2DM patients have not yet been demonstrated.

Systemic inflammation has been known to be responsible for the pathological conditions including cancer, heart diseases and cognitive decline (16-20). In the systemic inflammatory condition, white blood cells (WBC), particularly neutrophil and lymphocyte,, play an important role by releasing several pro-inflammatory cytokines and chemokines (21-24). Several studies demonstrated that the neutrophil to lymphocyte ratio (NLR) can be a prognostic marker for several inflammatory conditions (25-27). Recently, NLR has been used to be a predicted biomarker in several pathological conditions including cancers, cardiovascular diseases (16-18). The NLR parameter has been widely used in the detection of those diseases, since it was a non-invasive, inexpensive and applicable methods to use to detect systemic inflammation. In the brain, neuronal inflammation is also associated with the pathophysiology of neurodegenerative diseases, particularly AD (24, 28-32). Inflammation is related with brain oxidative stress resulting in neuronal cell deaths, which can further develop the cognitive impairment (32-34). Those findings suggest that NLR would be a prognostic marker in the memory dysfunction. However, the correlation between NLR and the cognitive impairment in patients with T2DM has not been examined. Therefore, the present study will investigate the correlation of NLR and the cognitive function in T2DM patients.

Despite of NLR ratio, lipopolysaccharide (LPS), an endotoxin which was commonly found in the outer cell wall of gram-negative bacteria, has been widely used to induce inflammatory models (35-37). The LPS-stimulation could induce the inflammation through the releasing of pro-inflammatory cytokines and also activate the nuclear factor-kappa B (NF-KB) mediated the mitogen-activated protein kinases (MAPKs) resulting in the regulation of cyclooxygenase-2 (COX2) and inducible nitric oxide synthases (iNOS) expressions which be responsible for inflammatory response (38-41). According to its important role in the systemic inflammation as well as the relationship of inflammation in cognitive impairment, the determination of LPS level in the circulation would be an important marker for early detection for cognitive impairment. However, this correlation has not yet been determined.

Type 2 Diabetes mellitus (T2DM)

Type 2 Diabetes mellitus (T2DM) has commonly known as the pathological condition characterize by high level of glucose the blood stream (hyperglycemia) and insufficient insulin level in accompanied with insulin resistance condition (42). T2DM has been known to be linked with both environmental factors such as obesity, sedentary lifestyle and physical inactivity as well as genetic factor (43). Summarized the association of risk factors and their underlying mechanisms in T2DM has demonstrated in figure 1.

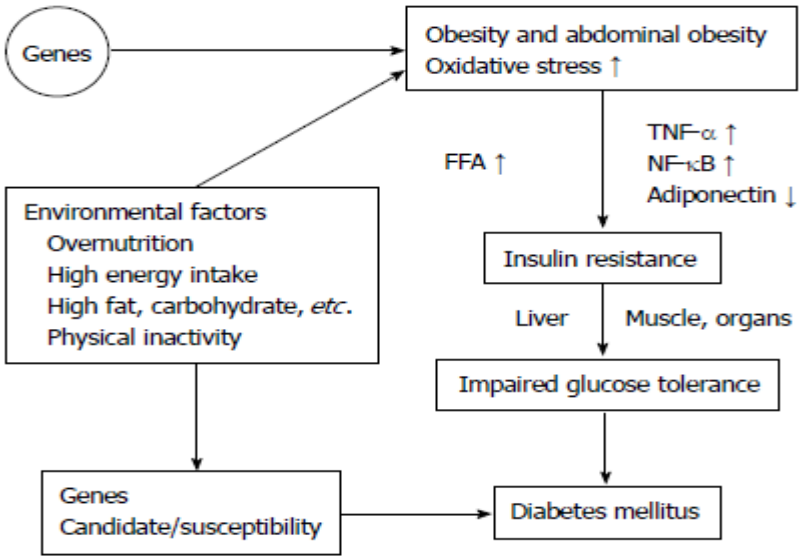

Figure 1. Summarized the pathogenesis of the T2DM (43). FFA: Free fatty acid; NF-ΚB: Nuclear factor-ΚB; TNF-α: Tumor necrosis factor α.

Patients are diagnosed as T2DM status when plasma glucose levels has reached at the diagnostic criteria in table 1 (1). Many of these T2DM individuals are at high risk for microvascular complications (e.g., nephropathy, retinopathy and neuropathy) and macrovascular complications (e.g., peripheral vascular disease, cerebrovascular disease and cardiovascular disease) (2). T2DM patients with good controlled plasma glucose levels demonstrated to delay the progression of microvascular and macrovascular complications (3, 4).

Table 1. Biochemical criteria for the diagnosis of diabetes, impaired glucose tolerance and impaired fasting glucose (1).

	Glucose concentration, mmol/l (mg/dl)		
	Plasma	Whole blood	
	Venous	Venous	Capillary
Diabetes mellitus			
Fasting and/or	≥ 7.0 (126)	≥ 6.1 (110)	≥ 6.1 (110)
2-h post-glucose load	≥ 11.1 (200)	≥ 10.0 (180)	≥ 11.1 (200)
Impaired glucose tolerance			
Fasting concentration (if measured) and	< 7.0 (126)	< 6.1 (110)	< 6.1 (110)
2-h post-glucose load	7.8–11.0 (140–199)	6.7–9.9 (120–179)	7.8–11.0 (140–199)
Impaired fasting glucose			
Fasting and	6.1–6.9 (110–125)	5.6–6.0 (100–109)	5.6–6.0 (100–109)
2-h (if measured)	< 7.8 (140)	< 6.7 (120)	< 7.8 (140)

The association of T2DM and cognitive impairment

Several studies have strongly shown that T2DM can be a risk factor for cognitive impairment and dementia (5, 6). Recent cross-sectional studies have demonstrated the lower cognitive performance in T2DM patients, compared with controls (7). In addition, the longitudinal studies have also indicated the acceleration of cognitive decline in T2DM individuals (8, 9). Biochemical parameters particularly in higher glucose level from T2DM and even non-T2DM subjects have been reported to be a risk factor for cognitive impairment or dementia (10, 11). Furthermore, various complications of metabolic and vascular disturbances such as disturbances of brain structures such as blood brain barrier, cerebral insulin signaling, insulin resistance, glucose toxicity and the formation of Advanced Glucose End-products (AGEs) have been implicated in the pathophysiology of cognitive impairment as shown in figure 2 (44, 45).

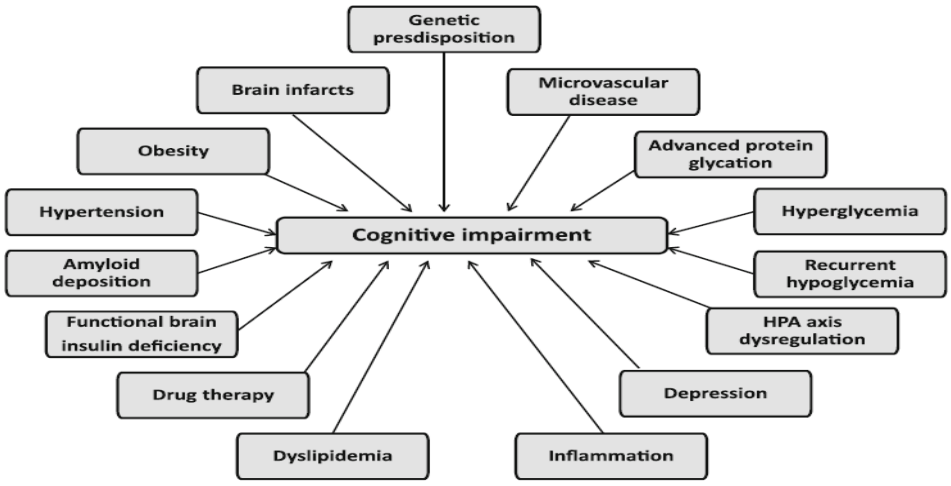

Figure 2. Potential causes of cognitive impairment in T2DM (45).

In addition, recent studies have been shown diabetes mellitus increased a risk factor of MCI (12-14). Previous study showed that the duration of diabetes, use of glucose-lowering medications, degree of glucose control should be the predictor of MCI (15). Although several factors in T2DM have been reported to associate with the cognitive decline, the relationship of these risk factors on the occurrence MCI has rarely been demonstrated. In addition, the prognostic markers for the early detection of MCI has not been investigated.

Mild cognitive impairment (MCI)

Mild Cognitive Impairment or MCI has been described as a state of cognitive dysfunction. This condition has been degenerated in cognition more than normal level (46) . The European Consortium on Alzheimer's disease defines the definition of MCI that showed in table 2.

Table 2. Definition of mild cognitive impairment (46).

Component	Definition
1	Memory problems, which are confirmed by the patient and by an informant
2	Decline in cognitive function in the past year compared to previous functioning reported by the patient and informant
3	Cognitive disorders are evidenced by clinical evaluation
4	Normal completion of activities of daily living
5	No diagnosis of dementia
Source: Portet et al (2006)	

MCI has been referred to the individuals with some cognitive impairment but the degree was insufficient to progress dementia (47, 48). Moreover, the individuals who have the MCI condition showed the increasing risk for developing the Alzheimer's disease (AD) (49, 50).

Most of previous clinical study of MCI reported that the 40-80% of individuals who met the criteria of MCI tends to develop the dementia or Alzheimer's disease, this finding was collected during a 5-years follow-up (51-53). Previous study reported that individuals with MCI also have vascular risk factors (54). Some patient has shown the occurrence of cerebral infarction or stroke which impact on the cognitive function (55, 56). Some study used a term of vascular cognitive impairment rather than MCI which indicated a high prevalence of vascular damage to the brain during cognitive decline (57).

In addition, recent studies have been shown diabetes mellitus increased a risk factor of MCI (12-14). Previous study showed that the duration of diabetes, use of glucose-lowering medications, degree of glucose control should be the predictor of MCI (15). Although several factors in T2DM have been reported to associate with the cognitive decline, the relationship of

these risk factors on the occurrence MCI has rarely been demonstrated. In addition, the prognostic markers for the early detection of MCI has not been investigated.

Possible mechanisms regarding the mild cognitive impairment

Several studies demonstrated the possible mechanisms involving the occurrence of mild cognitive impairment. In non-diabetic patients with amnestic mild cognitive impairment, they have been found the significant correlation of plasma insulin level in and the plasma amyloid-β level (58). The alteration of hippocampal precursor of nerve growth factor (pro-NGF)/NGF pathway which can contribute to amyloid-β accumulation has also been reported in MCI condition (59). Furthermore, some possible mechanisms such as loss of synapses in association with the neuronal apoptosis were found in MCI patients which could progress to Alzheimer's disease (60). The elevated pro-apoptotic protein expression of p53 caused by the oxidative modifications through lipid peroxidation was found in brain samples of MCI individuals (61, 62). In addition, recent study reported the role of oxidative-stress mediated mitochondrial dysfunction through activating extracellular signaling pathway in MCI condition (63). These findings indicated the occurrence of MCI condition through mitochondrial dysfunction, neuronal apoptosis, oxidative stress and the AD-like changes.

MCI patients also found the changes in orthostatic blood pressure behavior by demonstrating the systolic BP deficit and orthostatic intolerance [69, 70]. The Parkinson's disease (PD) patients who have MCI condition were found the abnormality in the brain by the occurrence of gray matter atrophy in several brain regions [71]. Moreover, several molecular mechanisms including brain apoptosis, oxidative stress condition were also reported during the MCI condition.

In addition, recent study showed the induction of mild cognitive impairment through advanced glycation end products (AGEs) pathway (64, 65). The patient who have MCI demonstrated a decreased soluble receptor for AGE (sRAGE) level as well as increased serum AGE-peptide (AGE-P) level. Diabetic patients also showed the association of C-reactive protein (CRP) and AGEs level with metabolic parameters particularly in glycosylated hemoglobin (HbA_{1C}) as well as negatively correlated with the Montreal Cognitive Assessment (MoCA) score which used to evaluate cognitive function (65). Moreover, several cytokines such as leptin and adiponectin were altered in the elderly diabetic patients whereas the inflammatory condition indicated by an increased interleukin-1 (IL-1) level was observed in these patients (66). Recently, an insulin signaling pathway has been also reported to play an important role in the pathogenesis of cognitive impairment caused by type 2 diabetes mellitus (T2DM) (67). They showed that the serum insulin-like growth factor (IGF)-1 to IGF binding protein (IGFBP)-3 molar ratio in MCI patients was significantly decreased and a significant negative correlation of this IGF-1/IGFBP-3 molar ratio and the Trail Making Test A and B (TMT-A and TMT-B) scores which indicated the executive function was found. These findings suggested the association of impaired insulin signaling pathway with the pathogenesis of MCI in T2DM populations. Furthermore, T2DM elderly patients who have MCI showed the inflammatory status by the increased of CRP, interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) compared to controls (66, 68) suggesting the induction of MCI through systemic inflammation.

Neutrophil to Lymphocyte ratio (NLR) as a novel prognostic marker for MCI

It has been known that systemic inflammation is responsible in several pathological conditions such as cancer, cognitive dysfunction and heart diseases (16-20). Systemic

inflammation could indicate by the alteration of biochemical markers. Under the pathological condition, white blood cells (WBC) including neutrophil and lymphocyte released several pro-inflammatory cytokines and chemokine (21-24). Since both neutrophil and lymphocyte are responsible for the inflammation occurring in the pathological condition, the neutrophil to lymphocyte ratio (NLR) can be a prognostic marker for these conditions (25-27). NLR has been used to be a predicted biomarker in several pathological conditions such as cancer, cardiovascular disease (16-18). NLR are widely used in the detection of these diseases since it was a novel method, non-invasive, inexpensive and easily applicable marker of inflammation.

In the brain, inflammation is also related to the pathophysiology of neurodegeneration, particularly in AD (24, 28-32). Inflammation can cause oxidative stress in brain leading to the neuronal cell deaths which can develop the memory impairment (32-34). These findings indicated that NLR would be a prognostic marker in the memory dysfunction although little known about this parameter and the development of cognitive decline has been reported. Recently, previous study firstly reported the evaluation of NLR in the AD (26). This study showed the NLR was significantly higher in AD patients than healthy controls. In addition, they found that the increasing of NLR was an independent variable for predicting AD suggesting that it could be a diagnostic marker in AD or cognitive dysfunction. However, this is a first study reporting the role of NLR in the diagnosis of memory dysfunction. Moreover, there are several limitations in this study such as each parameter measuring in this study were detected once, the relationship of NLR and comorbid conditions may have been confounded by some factor. The further studies are needed to support the benefit of this parameter in the detection of cognition function.

Lipopolysaccharide (LPS); a link in the T2DM

Lipopolysaccharide (LPS), an endotoxin which has been found in the outer cell wall from gram-negative bacteria that causes metabolic endotoxemia, has been widely used to induce inflammatory models (35-37). The LPS-stimulation can Kupffer cells can release pro-inflammatory cytokines and also regulate the cyclooxygenase-2 (COX2) and inducible nitric oxide synthases (iNOS) expressions through the activation of nuclear factor-kappa B (NF-KB) mediated the mitogen-activated protein kinases (MAPKs) (38-40). Consequently, COX-2 and iNOS expression have been known to responsible for inflammatory response (41).

The effect of diet on the dynamic modulation of microorganisms in the gut has been shown. Recently, several studies revealed an increasing in LPS levels has been found in leptin-deficient mice fed with a normal diet (69) as well as in subjects who increased their fat intake (70). They suggested that a change in either gut permeability or the proportion of gram-negative bacteria in gut were associated with an increased LPS in serum (69, 71) and this increase was associated with the degree of insulin resistance. In addition, recent studies also reported that prebiotics in obese mice modulated the intestinal microbiota on the intestinal barrier and decreased the high-fat diet-induced LPS endotoxemia and systemic inflammation (72, 73). All of these findings emphasized the role of microbiota particularly in LPS under the obese and even insulin resistance condition (Figure 3). In addition, since T2DM has been related with the development of cognitive impairment, the determination of LPS level in the circulation would be an important marker for the detection of MCI. However, this correlation has not yet been investigated.

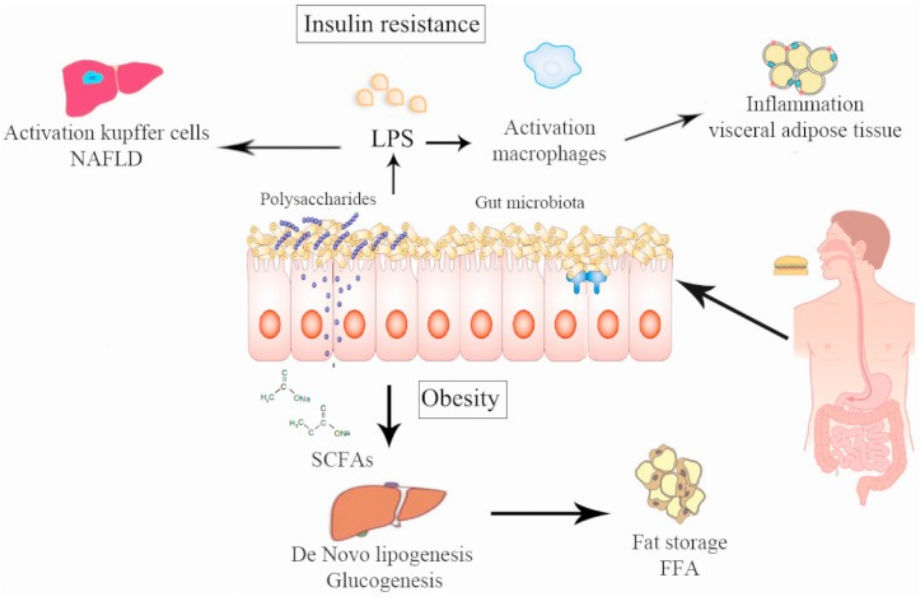

Figure 3. Roles of microbiota in the obesity and insulin resistance conditions (74).

Objectives

Aim 1: To determine whether the peripheral marker; NLR will be related with the occurrence of MCI in patients with T2DM.

Aim 2: To determine whether the peripheral marker; LPS will be related with the occurrence of MCI in patients with T2DM.

วิธีการทดลอง (Methods)

A cross-sectional study was used in this study for determination the risk factors associated with mild cognitive impairment among patients with T2DM.

Population

The subjects who have healthy (n = 30) and T2DM (n = 215) were conducted both from Maharaj hospital, Sriphum district and Saraphi hospital, Saraphi district, Chiang Mai, Thailand. A total number of 245 subjects in both genders who have age 45-65 years were conducted in this study.

Instrument

In the present study, we used the Mini-Mental State Examination (MMSE) test in Thai version. MMSE is a widely used screening tool for the identification of dementia (75). The MMSE begins with a graded assessment of orientation to place and time, for which a maximum of 10 points is possible. This is followed by testing two aspects of memory. A first is the immediate recall for three objects presented orally, followed by a serial sevens task which is interposed to assess attention, concentration, and calculation, and also to prevent the individual from rehearsing the three objects previously learned. A maximum of 11 points may be obtained in this section of the test. The final section surveys aphasia by testing functions of naming, repetition, understanding a three-stage command, reading, writing and copying a drawing. There is a maximum of 9 points which may be obtained on this section, for a total possible MMSE score of 30 points. Table 3 showed the score criteria for MMSE depending on the levels of education.

Table 3. MMSE criteria

Levels of education	MMSE Score (points)	
	Cut-off	total
No educated or illiterate	≤ 14	23
Elementary school educated	≤ 17	30
Higher than elementary school educated	≤ 22	30

However, previous studies have been shown that the MMSE has very poor sensitivity for individuals with MCI (75, 76). Therefore, the Montreal Cognitive Assessment (MoCA) test in Thai version for determine cognitive impairment was used in the present study. MoCA was designed as a rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. Time to administer the MoCA is approximately 10 minutes. The total possible score is 30 points; a score of 26 or above is considered normal.

Moreover, recent study has been shown that, the subject who has depression can cause cognitive impairment (14). Thus, the subjects who have depressive symptom were excluded from this study by using The Patient Health Questionnaire 9-item (PHQ-9), the depression test questionnaire. PHQ-9 is a self-reported that evaluated the depressive symptoms during the prior 2 weeks. Each item on the measure is rated on a 4-point scale (0=not at all; 1= several days; 2=more than half the days; and 3=nearly every day). The total score can range from 0 to 27, with higher scores indicating greater severity of depression as shown in table 4.

Table 4. PHQ-9 score criteria

Levels of depressive symptoms severity	PHQ-9 Score (points)
None	0-4
Mild depression	5-9
Moderate depression	10-14
Moderately severe depression	15-19
Severe depression	20-27

Moreover, the summarized experimental protocol for this study is shown in figure 4.

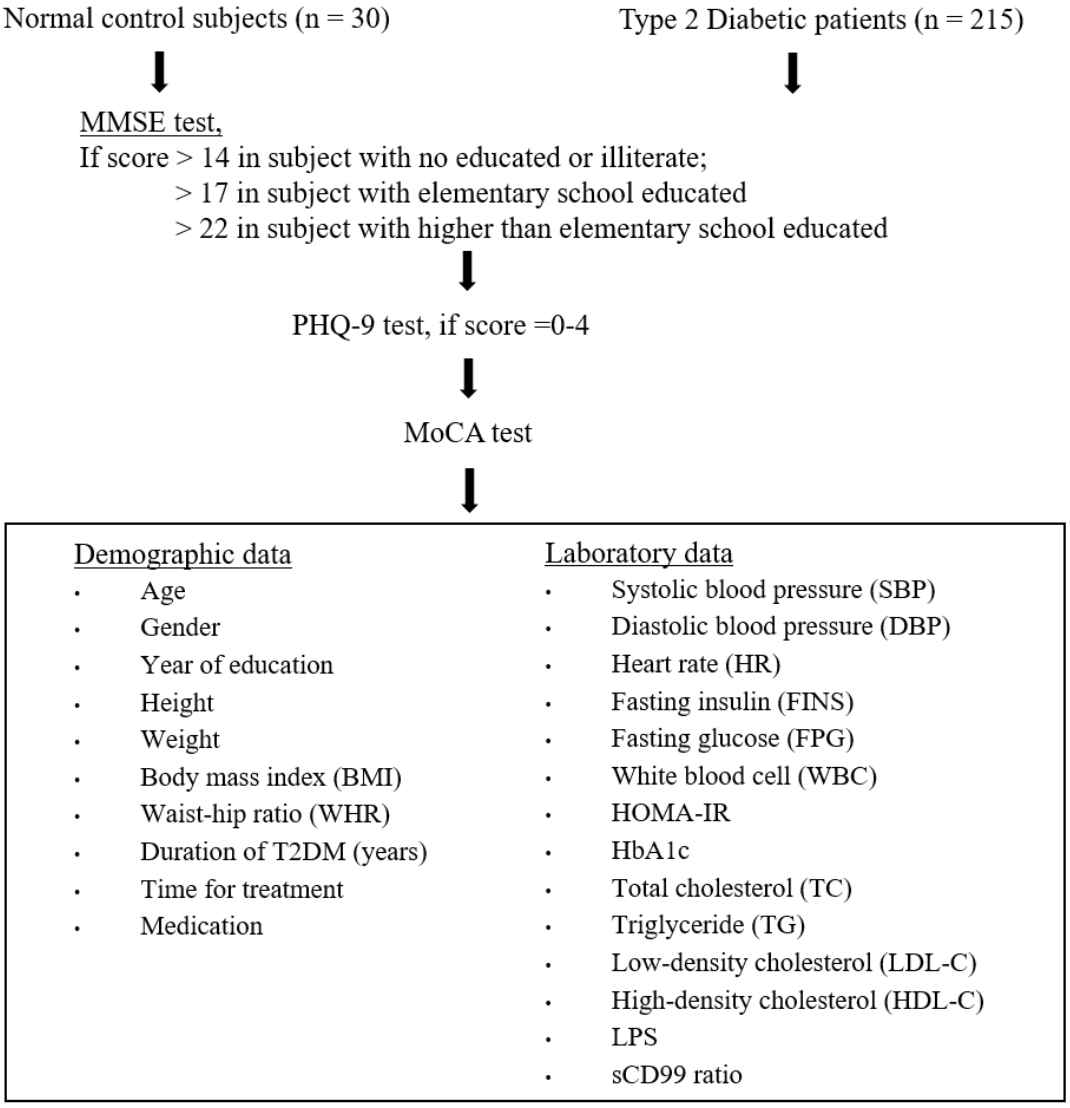

Figure 4. Summarized experimental protocols

Ethics

The Ethical approval was conducted from the Faculty of Medicine Ethics Committee, Chiang Mai University, Thailand.

Blood Biochemistry

After overnight fasting, blood samples were taken by venipuncture to assess serum levels of, fasting glucose, white blood cell, total cholesterol, triglycerides, LDL-C, HDL-C, HbA1C. All the parameters were measured in a centralized laboratory.

Determination of plasma insulin levels

Plasma insulin levels were determined by using Sandwich ELISA (LINCO Research, MO, USA).

Determination of insulin resistance (HOMA index)

Insulin resistance was assessed by Homeostasis Model Assessment (HOMA) which is calculated from fasting plasma insulin and fasting plasma glucose concentration. Higher HOMA index showed a higher degree of insulin resistance. The HOMA index was determined by the following equation:

$$\frac{[\text{fasting plasma insulin } (\mu U/ml)] \times [\text{fasting plasma glucose } (mmol/l)]}{22.5}$$

Determination of plasma LPS

Plasma LPS concentration was measured by a commercially available kit (Sigma Aldrich, Gillingham, UK) (14).

Determination of neutrophil-lymphocyte ratio (NLR)

The NLR was calculated from the differential count by dividing the absolute neutrophil count by the absolute lymphocyte count from WBC.

Determination of sCD99 ratio

Antibodies

Anti-CD99 mAb clone MT99/1 (isotype IgM) and MT99/3 (isotype IgG2a) were generated in our laboratory. Isotype-matched control mAb 13M (anti-phage protein; IgG2a) was produced in our laboratory (unpublished data). Horseradish peroxidase -conjugated goat anti mouse IgM (μ chain specific) antibody was purchased from Jackson Immuno Research Laboratory (West Grove, PA, USA).

Detection of soluble CD99 by ELISA

Anti-CD99 mAb clone MT99/3 or isotype matched control mAb clone 13M (10 μg/ml) was coated on ELISA plate in carbonate–bicarbonate coating buffer of pH 9.6 at 4°C overnight. After washing with 0.05% Tween-20 in phosphate buffer saline solution (0.05% Tween-PBS), the plates were blocked free surface with 2% bovine serum albumin-PBS for 1 hour. Serum (dilution at 1:50) was added into plates and incubated at 37°C for 1 hour. The plates were washed out for the excess antigen using 0.05% Tween-PBS. The detection antibody, anti-CD99 mAb clone MT99/1 (10 μg/ml) was added into plate and incubated at 37°C for 1 hour. After washing, horseradish peroxidase -conjugated goat anti mouse IgM (μ chain specific) antibody was added and incubated at 37°C for 1 hour. The reactions were detected using a TMB substrate and optical density was measured at 450 nm. The obtained signals were used to calculated soluble CD99 ratio as equation below:

Soluble CD99 ratio = signal obtained from anti-CD99 mAb (MT99/3) coated plate/
signal obtained from isotype matched control mAb (13M) coated plate

Data analysis

Demographic and metabolic parameter data were presented as mean± SEM. Independent t-test was used for comparison between two groups. A Pearson correlation method was used to determine the possible factors which correlated with an individual MoCA score. A univariate analysis was performed for the metabolic parameters that had a significant correlation with an individual MoCA score. In order to access the independent effects, the significant parameters from the univariate analysis in these patients were further collected into multivariate models and a multivariate analysis was performed. P-value <0.05 was considered as a statistical significance.

ผลการทดลอง (Results)

Demographic data and clinical parameters in all subjects

According to the numbers of control subjects, 1 subject from this group was excluded because of higher score than 4 points from PHQ-9 test which indicated a depression state. Metabolic parameters such as body mass index (BMI), the waist-hip ratio (WHR), fasting plasma glucose (FPG), glycated hemoglobin (HbA_{1C}), fasting insulin (FINS), HOMA-index, systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), fasting plasma glucose (FPG), fasting plasma insulin (FINS), total cholesterol (TC), triglyceride (TG), high-density cholesterol (HDL-C), low-density cholesterol (LDL-C) in T2DM group were significantly different when compared with control group (Table 5). Furthermore, neutrophil, lymphocyte, and the neutrophil-lymphocyte ratio (NLR) in T2DM patients were also changed.

Regarding the cognitive impairment, MMSE score in T2DM patients was decreased when compared with control (Table 5). MoCA score in this group was also reduced. Additionally, a prevalence of MCI was 93% in T2DM patients (200 out of 215 patients), while MCI prevalence in control group was 48% (14 out of 29) (Table 5). However, PHQ-9 score which was used to evaluate depression condition, between these groups was not different.

Table 5. Demographics of control and diabetic subjects

Demographic data	Control (n=29)	T2DM (n=215)
Male, n (%)	4(14%)	97 (45%)
Average Age (Years)	53 ± 1	55 ± 0
BMI (kg/m^2)	22.84 ± 0.57	26.18 ± 0.41*
WHR	0.84 ± 0.02	0.90 ± 0.01*
Years of Education (Years)	14.2 ± 0.6	6.8 ± 0.3*
Duration of DM (Years)	0 ± 0	8.9 ± 0.5*
SBP (mmHg)	119 ± 3	150 ± 13*
DBP (mmHg)	75 ± 2	78 ± 1*
HR (bpm)	77 ± 2	90 ± 5*
FPG (mmol/L)	95 ± 1	170 ± 6*
FINS (mIU/L)	21.7 ± 4.6	7.9 ± 0.7*
TC (mmol/L)	220 ± 7	180 ± 4*
TG (mmol/L)	120 ± 17	156 ± 8*
HDL-C (mmol/L)	56 ± 2	48 ± 1*
LDL-C (mmol/L)	142 ± 7	104 ± 4*
HbA_{1C} (%)	5.7 ± 0.2	8.2 ± 0.2*
Neutrophil (%)	53.0 ± 1.2	57.4 ± 0.7*
Lymphocyte (%)	38.0 ± 1.2	31.0 ± 0.6*
NLR	1.45 ± 0.09	1.98 ± 0.08*
MMSE (points)	27.8 ± 0.4	26.0 ± 0.2*
MoCA (points)	23.4 ± 0.7	19.6 ± 0.3*
PHQ-9 (points)	1.4 ± 0.4	1.7 ± 0.2
Prevalence of MCI (%)	48.27	93.02

BMI; body mass index, DBP; diastolic blood pressure, DM; diabetes mellitus, FINs; fasting plasma insulin, FPG; fasting plasma glucose, HbA_{1C}; glycated hemoglobin, HDL-C; high-density lipoprotein cholesterol, HR; heart rate, LDL-C; low-density lipoprotein cholesterol, MCI; mild-cognitive impairment, MMSE; mini-mental state examination, MoCA; Montreal cognitive assessment, NLR; neutrophil-lymphocyte ratio, T2DM; type-2 diabetes mellitus, TC; total cholesterol, TG; triglyceride, SBP; systolic blood pressure,. *p<0.05 vs. Control group.

Glycemic parameters and NLR showed a significant correlation with an individual MoCA score in control and T2DM subjects

Associative correlation between individual MoCA scores of control subjects and their metabolic parameters demonstrated that individual MoCA score was negatively correlated with blood glycemic profiles; HbA_{1C} (r = -0.517, *P* = 0.008) (Figure 5), whereas other parameters were not correlated with MoCA score (data not shown). On the other hand, table 6 demonstrated the correlation of demographic data and MoCA score from T2DM group. Metabolic profiles from T2DM patients indicated that body mass index (BMI) showed a strong positive correlation with MoCA score (r = 0.336, P = 0.000) (Figure 6A), while Fasting plasma glucose (FPG) from these patients had a negative correlation with MoCA score (r = -0.289, P = 0.006) (Figure 6B). In addition, age of these patients also indicated its negative correlation with MoCA score (r = -0.190, P = 0.003) (Figure 6C). Surprisingly, the neutrophil-lymphocyte ratio (NLR) showed a significant negative correlation with an individual MoCA score, (r = -0.402, *P* = 0.000, Figure 7).

Multivariate analysis for the association between MoCA score with various parameters of T2DM patients

Significantly correlated factors such as BMI, plasma FPG, Age, and the NLR were further used for univariate analysis and the MoCA score were set as the independent variable (Table 6). Multivariate analyses of the correlated metabolic parameters and MoCA score was also determined in order to discriminate the possible confounding factors (Table 5). After multivariate analysis was performed, the results demonstrated that only BMI and NLR were independently variable which were correlated with the MoCA score (Table 7). These findings suggest that NLR can be a prognostic indicator of poor cognitive performance in T2DM patients.

Table 6. Correlation of demographic data and MoCA score from T2DM patients

Demographic data	r	p (univariate)
Age	-0.190	0.013
BMI	0.336	0.000
WHR	-0.021	0.848
SBP	-0.050	0.781
DBP	0.207	0.248
HR	-0.220	0.262
FPG	-0.289	0.006
FINS	0.081	0.400
TC	-0.105	0.271
TG	-0.027	0.783
HDL-C	-0.022	0.820
LDL-C	-0.084	0.388
HbA_{1C}	-0.138	0.156
NLR	-0.402	0.000
serum LPS	0.044	0.659
sCD99 ratio	-0.079	0.343

BMI; body mass index, DBP; diastolic blood pressure, FINs; fasting plasma insulin, FPG; fasting plasma glucose, HbA_{1C}; glycated hemoglobin, HDL-C; high-density lipoprotein cholesterol, HR; heart rate, LDL-C; low-density lipoprotein cholesterol, LPS; lipopolysaccharide, NLR; neutrophil-lymphocyte ratio, T2DM; type-2 diabetes mellitus, TC; total cholesterol, TG; triglyceride, SBP; systolic blood pressure; sCD99; soluble cluster of differentiation 99, WHR; waist-hip ratio.

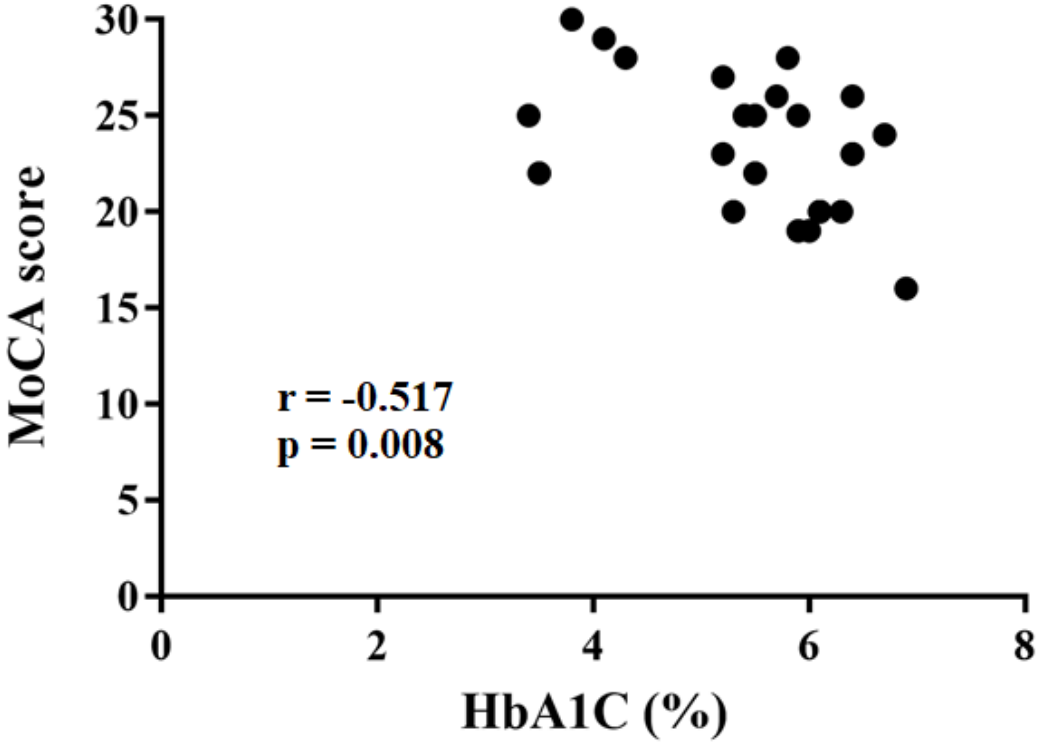

Figure 5

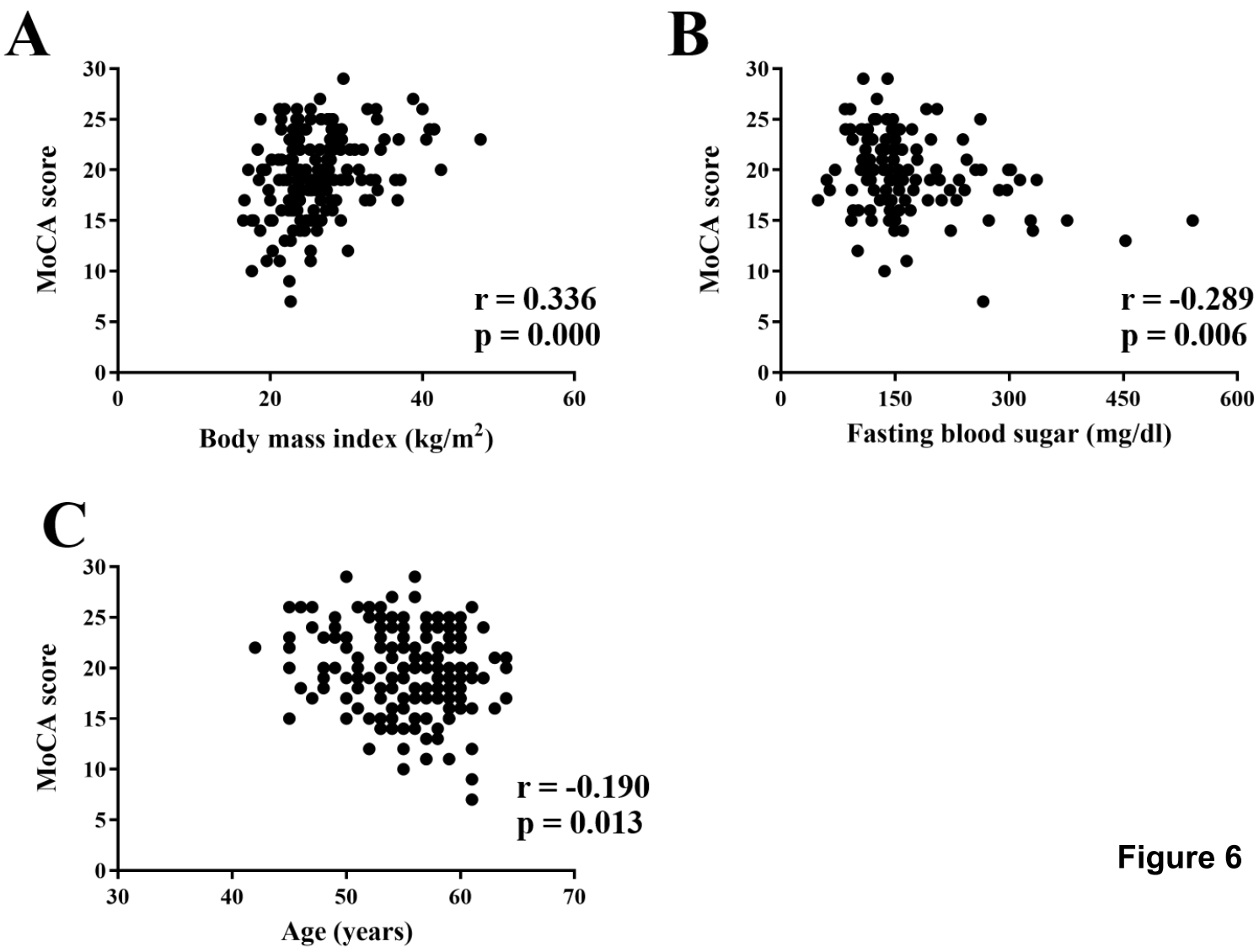
A
MoCA score
Body mass index (kg/m2)
r = 0.336
p = 0.000
B
MoCA score
Fasting blood sugar (mg/dl)
r = -0.289
p = 0.006
C
MoCA score
Age (years)
r = -0.190
p = 0.013

Figure 6

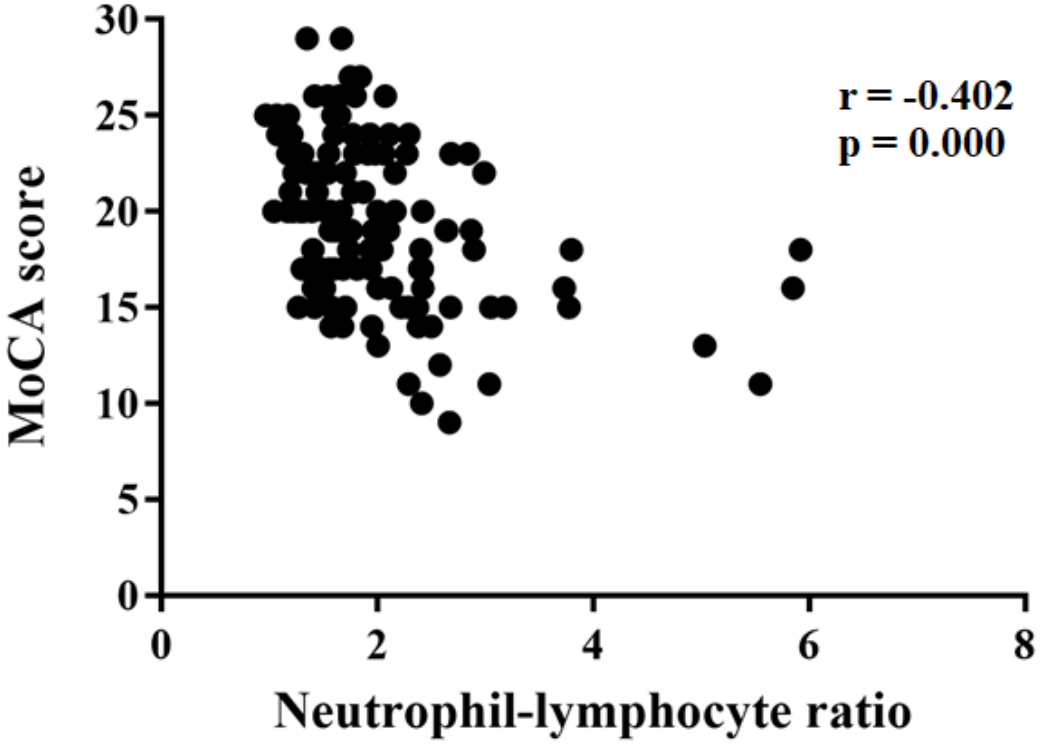
30
25
20
15
10
5
0
MoCA score
r = -0.402
p = 0.000
0
2
4
6
8
Neutrophil-lymphocyte ratio

Figure 7

Table 7. Multivariate analysis of significant variable from univariate analysis with MoCA score

Parameters	**B**	**p-value**	**VIF**
Age	-0.091	-0.949	1.041
BMI	**0.288**	**3.024**	**1.024**
FPG	-0.168	-1.709	1.088
NLR	**-0.260**	**-2.669**	**1.072**

BMI; body mass index, FPG; fasting plasma glucose, NLR; neutrophil-lymphocyte ratio, VIF; variance inflation factor.

บทวิจารณ์ (Discussion)

The major findings of this study are as follows 1) MCI was observed in control subjects and HbA1C is negatively related with MoCA in control group 2) Almost of T2DM demonstrated the MCI condition, 3) several metabolic parameters including Age, BMI, FPG, and NLR were shown to be correlated with MoCA score and 4) NLR and BMI level independently demonstrated the strongest correlation with the MoCA score in these T2DM patients.

Type 2 Diabetes mellitus (T2DM) has commonly known as the pathological condition characterize by high level of glucose the circulation (hyperglycemia) and insufficient insulin level in accompanied with insulin resistance condition (42). T2DM has been known to be linked with both environmental factors such as obesity, sedentary lifestyle and physical inactivity as well as genetic factor (43). Many of these T2DM individuals are at high risk for microvascular complications (e.g., nephropathy, retinopathy and neuropathy) and macrovascular complications (e.g., peripheral vascular disease, cerebrovascular disease and cardiovascular disease) (2). T2DM patients with good controlled plasma glucose levels demonstrated to delay the progression of microvascular and macrovascular complications (3, 4).

Several studies have strongly shown that T2DM can be a risk factor for cognitive impairment and dementia (5, 6). Recent cross-sectional studies have demonstrated the lower cognitive performance in T2DM patients, compared with controls (7). In addition, the longitudinal studies have also indicated the acceleration of cognitive decline in T2DM individuals (8, 9). Biochemical parameters particularly in higher glucose level from T2DM and even non-T2DM subjects have been reported to be a risk factor for cognitive impairment or dementia (10, 11). Furthermore, various complications of metabolic and vascular disturbances such as disturbances

of brain structures such as blood brain barrier, cerebral insulin signaling, insulin resistance, glucose toxicity and the formation of Advanced Glucose End-products (AGEs) have been implicated in the pathophysiology of cognitive impairment (44, 45).

Cognitive impairment can be categorized as ranging from mild to severe. With mild cognitive impairment, individuals might begin to notice changes in their cognitive functions, although they are still able to do their daily-life activities. In contrast, a severe degree of cognitive impairment leads to a loss of an individual's ability or skills to understand the meaning or importance of their surroundings, hence they are incapable of live independently. Moreover, a severe degree of cognitive impairment can lead to the development of AD. In addition, patients with cognitive impairment have a higher risk of being hospitalized compared with individuals who are hospitalized for other conditions.

In addition, recent studies have been shown diabetes mellitus increased a risk factor of mild cognitive impairment (MCI) (12-14). Previous study showed that the duration of diabetes, use of glucose-lowering medications, degree of glucose control should be the predictor of MCI (15). Although several factors in T2DM have been reported to associate with the cognitive decline, the relationship of these risk factors on the occurrence MCI has rarely been demonstrated. In addition, the prognostic markers for the early detection of MCI has not been investigated.

In patients with obesity, peripheral inflammation was observed which is thought to be caused by the production of pro-inflammatory adipokines, cytokines and chemokines. Moreover, previous studies reported that inflammation can lead to the progression of cognitive dysfunction and AD (77, 78). A high level of plasma adiponectin was found to be a risk factor for causing dementia and AD (79). Additionally, T2DM elderly patients who have MCI showed the

inflammatory status by the increased of CRP, interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) compared to controls (66, 68) suggesting the induction of MCI through systemic inflammation.

Brain inflammation is also responsible for causing neurodegenerative diseases (24, 28-32). Currently, NLR has been used as a biomarker to predict several pathological conditions such as cancer and cardiovascular disease (16-18). NLR are widely used as it has the advantage of being a novel and non-invasive method of assessing these conditions. These findings imply that NLR may be a prognostic marker for cognitive impairment, although there is no study reporting this relationship.

In the present study, we found several factors that correlated with the development of MCI condition in T2DM patients, which include Age, BMI, and FPG. Interestingly, we found that systemic inflammation was also found to be related with MCI, as indicated by the correlation of neutrophil-lymphocyte ratio (NLR) with MoCA score. This finding suggests that systemic inflammation could be one of the underlying mechanisms for the induction of cognitive impairment in these diabetic patients.

Notably, we also found that the strongest correlation found among all parameters was between NLR and MoCA cognitive score. Supporting this finding, multivariate analysis indicated that only NLR and BMI independently provided a significant correlation with an individual MoCA score. Several studies reported that NLR may be a good predictor of cognitive deficit in several pathological conditions such as carotid endarterectomy and Alzheimer's disease (19, 80, 81). This demonstrates that NLR may be a novel prognostic marker for MCI found in T2DM, however further studies are still needed to support this finding.

However, there were a number of limitations in the present study: 1) the enrolled patients in this study received different therapies for the treatment of DM and dyslipidemia, which might have led to a lack of correlation between some of their metabolic parameters and MoCA score and 2) since some of demographics such as history of T2DM was obtained by subjective examination, this might affect the association of these profiles with MoCA.

In conclusion, the present study demonstrated that MCI is common in T2DM patients. There were several clinical parameters correlated with MCI displayed in MetS patients. Interestingly, we also found that NLR was closely correlated with MCI, suggesting the beneficial role of this parameter as a novel prognostic marker for the evaluation of cognitive impairment in the T2DM before the development of other severe complications.

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. **ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า)**

ยังอยู่ในระหว่างการเขียน manuscript เพื่อตีพิมพ์ในวารสารระดับนานาชาติต่อไป

2. **การนำผลงานวิจัยไปใช้ประโยชน์**

2.1 **เชิงสาธารณะ (มีเครือข่ายความร่วมมือ/สร้างกระแสความสนใจในวงกว้าง)**

ได้มีการนำเสนอผลงานวิจัยในงานประชุมทั้งในระดับชาติ และนานาชาติ โดยได้เผยแพร่ความรู้เกี่ยวกับตัวพยากรณ์ที่เป็นไปได้ต่อการเกิดภาวะความจำบกพร่องแบบต่ำ ๆ ในผู้ป่วยโรคเบาหวานชนิดที่ 2 ซึ่งจะช่วยให้มี parameter ที่มีประสิทธิภาพอีกประเภท ที่เป็นประโยชน์สำหรับแพทย์ และ พยาบาล สำหรับใช้คัดกรองความเสี่ยงต่อการเกิดความจำเสื่อมโดยเฉพาะอย่างยิ่ง โรคอัลไซเมอร์ ซึ่งพบได้มากในผู้ป่วยโรคเบาหวานชนิดที่ 2

2.2 **เชิงวิชาการ (มีการพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่)**

ยังอยู่ในระหว่างการเขียน manuscript เพื่อตีพิมพ์ในวารสารระดับนานาชาติต่อไป

3. **อื่น ๆ (เช่น หนังสือ การจดสิทธิบัตร)**

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