The Differences of Serum Metabolites Between Patients With Early-Stage Alzheimer’s Disease and Mild Cognitive Impairment

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Background: Mild cognitive impairment (MCI) is regarded as a transition phase between normal aging and Alzheimer’s disease (AD). Identification of novel and non-invasive biomarkers that can distinguish AD at an early stage from MCI is warranted for therapeutic and support planning. The goal of this study was to identify the differences of serum metabolomic profiles between MCI and early-stage AD, which could be potential non-invasive biomarkers for early diagnosis of AD.

Methods: The subjects enrolled in the study were classified into two diagnostic groups: MCI (n = 40) and early-stage AD (n = 40). Targeted metabolomics analysis of serum samples was performed using the Biocrates Absolute-IDQ P180 kit. Targeted metabolic data were analyzed by TargetLynx, and MetIDQ software was applied to integrate the metabolites by automated calculation of metabolite concentrations.

Results: The datasets of targeted metabolite analysis were analyzed by the orthogonal-projection-to-latent-structure–discriminant-analysis (OPLS-DA) model. The OPLS-DA score plots demonstrated considerable separation between the MCI and early-stage AD patients. The levels of pimelylcarnitine, putrescine, SM (OH) C24:1, and SM C24:0 were significantly lower, whereas the levels of acetylornithine, methionine sulfoxide, and PC ae C44:3 were significantly higher in early-stage AD patients compared with MCI patients. Receiver operating characteristic curve analysis of a combination of three lipid metabolites [SM (OH) C24:1, SM C24:0, and PC ae C44:3] showed an acceptable discrimination between the early-stage AD and MCI patients (area under the curve = 0.788).

Conclusions: Our results characterized the differences of serum metabolic profiles between MCI and early-stage AD patients. The positive findings from this study indicate that the minimally invasive method of blood sampling may help to identify patients with AD at an early stage from those with MCI.

Keywords: Alzheimer’s disease, mild cognitive impairment, dementia, metabolomics, P 180 kit, serum metabolite
INTRODUCTION

Alzheimer’s disease (AD) is a neurodegenerative disease of the brain, characterized by senile plaques, neurofibrillary tangles, selective loss of neurons and synapse, inflammation and glial responses, and vascular alterations (1). The clinical features of AD include memory impairment, cognitive decline, and behavior changes (2). Mild cognitive impairment (MCI) is regarded as a transition period between age-appropriate memory changes and AD (3). The presentation of MCI includes cognitive deficits, mainly in memory functions, with preservations of independence in everyday activities and does not fulfill the criteria of AD, other dementia disorders, or other mental diseases. MCI is associated with an increased risk of progression to AD (4). The outcomes of the MCI state are diverse and with several possibilities, which even include improving back to normal cognition (5).

Recent research has focused on searching for sensitive and specific biomarkers for the early diagnosis of AD and for identifying MCI patients who will progress to AD from stable MCI or healthy elderly control subjects (6–12). The ideal biomarkers of AD should be non-invasive and could reflect the disease-related biological processes, such as blood samples. Other non-invasive biomarker studies for AD have been done in saliva (13, 14) or tear fluid (15). However, the comparisons of saliva and serum analyses revealed the limitations in using saliva for biomarker discovery (16). There is growing evidence that a single biomarker cannot have enough sensitivity and specificity for AD diagnosis since different research groups identified different biomarkers, and some results cannot be reproduced (6, 17, 18). Therefore, the ideal non-invasive biomarkers for detecting AD in very early stage remain uncertain.

Metabolomics is a discipline specially used in the global study of small molecules in cells, tissues, and biofluids. Concentration changes of specific groups of metabolites may reflect the state of disease progression, and metabolomics has become a powerful tool for biomarker development (10). Currently, technologies of metabolomics have allowed comprehensive and quantitative investigation of different metabolites in various diseases (19). A systemic review by Jiang et al. demonstrated that the concentrations of several metabolites, including lipids (higher phosphatidylcholines, sphingomyelins (SMs), and lysophosphatidylcholine and lower docosahexaenoic acid and high-density lipoprotein subfractions), amino acids (lower branched-chain amino acids, taurine, and higher glutamate, glutamine, and anthranilic acid), were associated with cognitive decline and the incidence or progression of dementia (20). In addition, Fleszar et al. showed that L-arginine/NO pathway in blood was altered in AD and vascular dementia (21). And dysregulated arginine metabolism in urine may serve as a diagnostic biomarker for old MCI patients (22). Moreover, Kim et al. reported that primary fatty amides in plasma were associated with brain amyloid burden, hippocampal volume, and memory (23).

The aim of the present study was to analyze the metabolome of serum samples from MCI and early-stage AD patients. We quantitatively analyzed 40 acylcarnitine metabolites, 21 amino acids, 19 biogenic amines, 15 sphingolipids, and 90 glycerophospholipids using the Absolute-IDQ P180 kit (Biocrates Life Sciences AG, Innsbruck, Austria). We here demonstrated that the concentrations of several serum metabolites were significantly different between patients of MCI and early-stage AD, which may serve as a potential non-invasive biomarker for distinguishing MCI patients who will progress to early-stage AD from MCI patients who will remain in the MCI stage.

MATERIALS AND METHODS

Participants

Patients who visited an outpatient clinic of neurology for memory impairment or behavior change from July 2015 to September 2017 were potential participants of this study. Evaluation of medical history and physical and neurological examinations were performed by two experienced neurologists. Blood tests for a dementia survey that included cortisol levels, thyroid function, syphilis screen, vitamin B12, and folate level and a brain imaging study that included computed tomography (CT) or magnetic resonance imaging (MRI) were arranged as routine survey for dementia. Patients with abnormalities in blood tests for the dementia survey were excluded. The brain imaging study was evaluated by an experienced neuroradiologist, and those with abnormal brain lesions such as old cerebrovascular insults, tumor, encephalomalacia, marked white matter lesions, or other brain lesions were excluded from this study. Patients with a history of psychiatric disorder, depression, old stroke, severe renal or liver dysfunction, or malignancy were also excluded. Mini-Mental State Examination (MMSE) (24), Clinical Dementia Rating (CDR) (25), and Cognitive Abilities Screening Instrument (CASI) (26) were used for dementia evaluation and were performed by an experienced neuropsychologist. Patients were excluded from the study if (1) age is <65 years; (2) they received education for <6 years; (3) they had abnormality in the blood test for the dementia survey that included cortisol levels, thyroid functions, syphilis screen, vitamin B12, and folate levels; (4) CT or MRI show abnormal brain lesions such as old cerebrovascular insults, tumor, encephalomalacia, marked white matter lesions, or other brain lesions; (5) they had a history of psychiatric disorder, depression, old stroke, or malignancy; (6) they had abnormality of renal or liver function tests; (7) they had poorly controlled diabetes mellitus (glycohemoglobin ≥7%); (8) they had an MMSE score of 30 or <20 and a CDR score of 0 or >1; (9) they already used a cholinesterase inhibitor. To reduce the influence of hypnotic drugs on metabolomics profiles in these patients, we collected blood samples from the patients after prohibiting eatables and medication intake for at least 8 h in our study.

MCI was diagnosed using the criteria originally proposed by the Mayo Clinic Alzheimer’s Disease Research Center (27). The MCI criteria were as follows: (1) memory complaint by patient, family, or physician; (2) normal activities of daily living; (3) normal global cognitive function; (4) objective impairment in memory or in one other area of cognitive function; (5) a CDR score of 0.5 and an MMSE score around 26–29 (28); and (6) absence of dementia. The diagnosis of AD was based
on the criteria of the National Institute of Neurologic and Communicative Disorders and Stroke (29). Patients with a CDR score of 1 and an MMSE score around 20–25 were regarded as early-stage AD and were recruited into the study.

The study was designed and carried out in accordance with the principles of the Declaration of Helsinki and with approval from the Ethics Review Board of Chang Gung Memorial Hospital (IRB 104-4769B and IRB 104-6261B). All subjects gave written informed consent.

**Metabolomic Analysis**

Targeted metabolomic analysis of serum samples was performed using the Biocrates Absolute-IDQ P180 kit (Life Science AG, Innsbruck, Austria). The serum samples were processed as per the manufacturer instructions and analyzed on a triple-quadrupole mass spectrometer (Waters, Milford, CT, USA). As part of the quality control, three concentrations of quality controls were included in the kit. Targeted metabolic data were analyzed by TargetLynx (Waters, Milford, CT, USA), and MetIDQ software (Biocrates, Innsbruck, Austria) was applied to integrate the metabolites by automated calculation of metabolite concentrations. Metabolites with the concentration below the limit of detection were excluded.

**Statistical Analysis**

Continuous variables were expressed as mean ± standard deviation (SD) if the values were normally distributed or median and interquartile range if the values were not normally distributed. Categorical variables were expressed as a number, or percentage, for each item. The MCI and early-stage AD patient groups were compared using chi-square, Mann–Whitney U, or Student t-test. To maximize identification of differences in metabolic profiles between groups, the orthogonal-projection-to-latent-structure–discriminant-analysis (OPLS-DA) model was applied using the SIMCA-P software (version 13.0, Umetrics AB, Umea, Sweden). The variable importance in the projection (VIP) value of each variable in the model was calculated to indicate its contribution to the classification. A higher VIP value represented a stronger contribution to discrimination among groups. VIP > 1.5 were considered significantly different. All statistical analyses were two-sided and performed using IBM SPSS statistics 19.0 software (Armonk, NY, USA) for Windows. A P < 0.05 was considered significant.

**RESULTS**

**Patient Characteristics**

A total of 80 participants included 40 MCI and 40 early-stage AD patients. The characteristics and laboratory data of the included patients were shown in Table 1. The early-stage AD patients were significantly older than the MCI patients (P < 0.001). The durations from symptom onset to diagnosis were significantly longer in early-stage AD patients as compared with MCI patients (P < 0.001). Upon comorbidities, the prevalence of hyperlipidemia was significantly higher in MCI patients as compared with early-stage AD patients (P = 0.03). The percentage of hypnotic drug usage was significantly higher in MCI patients (P = 0.017). According to laboratory data, early-stage AD patients had lower levels of hemoglobin, alanine transaminase, and total cholesterol as compared with MCI patients (P = 0.018, <0.001, and 0.006, respectively).

**Serum Metabolomics Analysis**

To evaluate the differences of serum metabolites between MCI and early-stage AD patients, serum from 40 MCI and 40 early-stage AD patients were subject to targeted metabolite analysis, and datasets were analyzed by the OPLS-DA model. The OPLS-DA score plots demonstrated considerable separation between the MCI and early-stage AD patients (Figure 1A). The metabolites responsible for the discrimination between these two groups (those with VIP > 1.5) are listed in Table 2. The levels of pimelylcarnitine, putrescine, SM (OH) C24:1, and SM C24:0 were significantly lower, whereas the levels of acetylornithine, methionine sulfoxide (Met-SO), and PC ae C44:3 were significantly higher in early-stage AD patients as compared with MCI patients (Table 2, Figures 1B–H).

The receiver operating characteristic (ROC) curve analysis of seven individual metabolites did not show good discrimination between MCI and early-stage AD patients [area under the curve (AUC) demonstrated in Table 2]. ROC curve analysis of a combination of these seven metabolites showed good discrimination of early-stage AD and MCI patients (AUC = 0.811). However, ROC curve analysis of a combination of three lipid metabolites [SM (OH) C24:1, SM C24:0, and PC ae C44:3] showed an acceptable discrimination between MCI and early-stage AD patients (AUC = 0.788, Figure 2). The finding supported the importance of dysregulation of brain lipid metabolisms in cognitive decline.

**DISCUSSION**

In the current study, we found that the levels of several serum metabolites had significant differences between patients of MCI and early-stage AD, which might be potential biomarkers for distinguishing MCI patients who will progress to early-stage AD from stable MCI patients. The targeted metabolite analysis using the Absolute-IDQ P180 kit revealed that the levels of pimelylcarnitine, putrescine, SM (OH) C24:1, and SM C24:0 were significantly lower, whereas the levels of acetylornithine, Met-SO, and PC ae C44:3 were significantly higher in sera of early-stage AD patients as compared with MCI patients.

Previous studies have demonstrated that the metabolites of cerebrospinal fluid could predict dementia development. However, the procedure of collecting cerebrospinal fluid is relatively invasive (30, 31). The diagnostic value of less invasive biomarkers, such as metabolites of blood or saliva samples, remains controversial. Previous blood metabolite studies of AD suggested that some lipids (32, 33) or metabolites (10, 34) could predict the progress of AD. In addition, Zheng et al. found that cognitively healthy adults and MCI patients could be differentiated with each other by the analyses of metabolites identified from saliva samples (14). However, other studies did not reveal a clear group separation for saliva samples among dementia and normal patients but found significant differences...
|                         | Mild cognitive impairment (n = 40) | Early-stage Alzheimer's disease (n = 40) | Odds ratio (95% CI) | P-value |
|-------------------------|-----------------------------------|-------------------------------------------|---------------------|---------|
| Age (years)             | 68 (67–72)                        | 77 (73–81)                                | <0.001*            |
| Female                  | 44 (67.7%)                        | 25 (65.8%)                                | 0.83 (0.36–1.91)   | 0.415   |
| Duration of symptoms (months) | 12 (6–24)             | 24 (12–36)                                | <0.001*            |
| MMSE                    | 28 (26–29)                        | 22 (20–24)                                | <0.001*            |
| CDR                     | 0.5 (0.5–0.5)                     | 0.5 (0.5–0.5)                             | <0.001*            |
| CASI                    | 86 (80–91)                        | 65 (60–72)                                | 0.005*             |
| Comorbidity             |                                   |                                           |                     |
| Hypertension            | 33 (50.8%)                        | 21 (55.3%)                                | 0.84 (0.37–1.86)   | 0.407   |
| Diabetes mellitus       | 11 (16.9%)                        | 11 (28.9%)                                | 0.50 (0.19–1.30)   | 0.118   |
| Hyperlipidemia          | 36 (55.4%)                        | 13 (34.2%)                                | 2.39 (1.04–5.47)   | 0.03*   |
| Coronary artery disease | 9 (13.8%)                         | 5 (13.2%)                                 | 1.06 (0.33–3.43)   | 0.586   |
| Chronic kidney disease  | 1 (1.5%)                          | 3 (7.9%)                                  | 0.16 (0.02–1.82)   | 0.141   |
| Gout                    | 3 (4.8)                           | 4 (10.5)                                  | 0.41 (0.09–1.95)   | 0.225   |
| Insomnia                | 24 (36.9%)                        | 10 (26.3%)                                | 1.64 (0.68–3.95)   | 0.188   |
| Hypnotic drug use       | 22 (33.8%)                        | 5 (13.2%)                                 | 3.38 (1.16–9.86)   | 0.017*  |
| Antidepressant use      | 4 (6.2%)                          | 1 (2.6%)                                  | 2.43 (0.26–22.5)   | 0.388   |
| Laboratory data         |                                   |                                           |                     |
| TSH, nIU/ml             | 1.78 (1.07–2.34)                  | 1.42 (1.00–2.51)                          | 0.420              |
| Free T4, mg/dl          | 1.14 (0.99–1.26)                  | 1.13 (1.02–1.29)                          | 0.940              |
| Cortisol, µg/dl         | 9.5 (6.9–12.5)                    | 9.1 (6.4–10.7)                            | 0.146              |
| Vitamin B12, pg/ml      | 610.3 (458.3–1,000.45)            | 593.95 (436.95–1,242.5)                   | 0.954              |
| Folate, mg/ml           | 9.64 (7.28–12.93)                 | 10.97 (7.65–16.42)                        | 0.284              |
| WBC, 1,000/µl           | 5,700 (4,950–7,000)               | 6,150 (5,275–7,850)                       | 0.270              |
| Hemoglobin, g/dl        | 13.6 (12.6–14.5)                  | 13.1 (12.1–13.6)                          | 0.018*             |
| Platelet, 1,000/µl      | 217.0 ± 54.4                      | 220.3 ± 59.6                              | 0.774              |
| Sugar, mg/dl            | 102 (93–111)                      | 101 (92.5–125.3)                          | 0.523              |
| Glycohemoglobin, g/dl   | 5.8 (5.6–6.2)                     | 5.95 (5.70–6.35)                          | 0.153              |
| AST, U/L                | 23 (20–29)                        | 23 (18–27)                                | 0.409              |
| ALT, U/L                | 23 (19–29)                        | 16 (14–23)                                | <0.001*            |
| Total cholesterol, mg/dl| 203 (180.5–233)                   | 182 (153.5–204)                           | 0.006*             |
| Triglyceride, mg/dl     | 123 (87.5–178)                    | 99 (66.5–130.8)                           | 0.051              |
| LDL-C, mg/dl            | 119 (107–146)                     | 111 (91–134)                              | 0.066              |
| HDL-C, mg/dl            | 47 (41–59)                        | 48 (40–58)                                | 0.632              |
| Uric acid, mg/dl        | 6.0 (5.2–6.5)                     | 5.9 (5.0–6.2)                             | 0.425              |
| hs-CRP, mg/L            | 0.8 (0.3–2.0)                     | 0.8 (0.2–2.4)                             | 0.891              |

CI, confidence interval; MMSE, Mini-Mental Status Examination; CDR, Clinical Dementia Rating; CASI, Cognitive Abilities Screening Instrument; TSH, thyrotropin; T4, thyroxine; WBC, white blood cells; AST, aspartate aminotransferase; ALT, alanine transaminase; BUN, blood urea nitrogen; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein.

Data are presented as mean ± standard deviation, median (interquartile range), or n (%). * P < 0.05, Student t, Mann–Whitney U, or chi-square test.

for serum samples (16). This finding suggests that blood samples are more suitable for non-invasive biomarker discovery of AD as compared with saliva.

Our results showed that the serum level of pimelylcarnitine, one kind of acylcarnitine, was significantly lower in early-stage AD patients as compared with MCI patients. Pimelylcarnitine had been reported to be associated with sleep deprivation (35), migraine (36), and type II diabetes mellitus (37). Acylcarnitines are formed from the conversion of acyl-CoA species by carnitine palmitoyltransferase (CPT) I (38). Then acylcarnitines are transported into the mitochondrial matrix by the carnitine acylcarnitine translocase, a mitochondrial inner membrane transporter. Finally, the enzyme CPT2 reconverts acylcarnitines back into free carnitine and long-chain acyl-CoAs (39). There is growing evidence that a net efflux of acylcarnitine species from the mitochondria into the cytosol and finally into plasma is crucial in the conditions of impaired fatty acid oxidation, because it could prevent the potentially toxic acyl-CoA intermediates from accumulating in the mitochondrion (40). Therefore, the changes of plasma or urinary acylcarnitine profiles have...
been used to detect fatty acid and amino acid oxidation disorders (41–43).

Our results found that the serum level of Met-SO was significantly higher in early-stage AD patients as compared with MCI patients. Met-SO, an oxidized form of methionine, is known to be an indicator of systemic oxidative stress since methionine is particularly susceptible to oxidation by reactive oxygen species (44, 45). To protect the cells from these toxic radicals, the Met-SO reductase system could reduce Met-SO to methionine (46). In a previous AD study, oxidative stress is one of the earliest consequences of toxic insults mediated by soluble amyloid β-protein (Aβ) oligomers (47). The Met-SO has been found to comprise 10–50% of Aβ in amyloid plaques of AD brain (48). As the oxidative stress is increased with advanced age and dementia stage while the activity of the methionine sulfoxide reductase system and other antioxidants are decreased, the level of Met-SO should be higher in more severe AD stages. This might be able to explain why the level of Met-SO is higher in early-stage AD patients when compared to MCI patients in this study.

Our results revealed that the serum level of putrescine, the precursor of spermidine, was significantly lower in early-stage AD patients as compared with MCI patients. Hydrolysis of L-arginine by arginase generates L-ornithine, which is decarboxylated by ornithine decarboxylase to form putrescine. Putrescine can also be produced by hydrolysis of agmatine, which is formed from L-arginine by arginine decarboxylase. Putrescine is alkylated by the decarboxylated S-adenosyl-methionine, which regularly serves as the major methyl group donor for methyl transfers (49). There is growing evidence that arginine metabolism may be associated with the development of AD and other dementia (50–52). Furthermore, in a recent study by Zhang et al., reduced urinary arginine levels were noted in patients with amnestic MCI (22).

In our results, the serum levels of SM (OH) C24:1 and SM C24:0 were significantly lower in early-stage AD patients as compared with MCI patients. In the brain, the proper balance of sphingolipids is essential for normal neuronal function, and subtle changes in sphingolipid balance may be intimately involved in neurodegenerative diseases, including AD. SMs are major components of cell membranes and are especially enriched in the central nervous system. Clinical, laboratory, and animal studies thus far suggest that perturbations in SM may contribute to the pathophysiology of AD, particularly the formation of amyloid-beta, associated amyloid plaques, and neurodegeneration (53). Previous study revealed that higher levels of SM (OH) C22:1 were significantly associated with lower risk of dementia (7). Besides, higher concentrations of three other SMs [SM C26:0, SM (OH) C22:2, and SM (OH) C24:1] might have a trend of lower prevalence of dementia, though these associations did not reach statistical significance (7). Our results are in agreement with previous findings on the link between AD and SMs (7, 53, 54). In the brain, the proper balance of sphingolipids is important for maintaining normal neuronal function. Therefore, the process of sphingolipid metabolism is altered in the early stage of AD and contributes to the neuropathological disturbances of AD, which include Aβ production, tau protein formation, and neurodegeneration (55).

We found that N-acetylspermidine was significantly higher in early-stage AD as compared with MCI patients. N-Acetylspermidine, an intermediate in arginine metabolism, is a minor component of deproteinized human blood (56). It has been linked to chronic kidney disease and been regarded as a potential biomarker for kidney function in humans since a previous study found that higher levels of N-acetylspermidine were associated with lower estimated glomerular filtration rate (57). However, no previous study has linked N-acetylspermidine to neurodegenerative diseases except one recent study revealing that there is a relationship between decreased N-acetylspermidine level and haloperidol exposure (58). Therefore, a causal relationship between increased N-acetylspermidine levels
and early-stage AD, as observed in our study, is difficult to speculate upon.

In addition, the level of PC ae C44:3, a metabolite of glycerophospholipids, was significantly higher in early-stage AD patients as compared with MCI patients. Glycerophospholipids, one kind of brain lipids, constitute a varied group of molecules with important brain functions (59). Several studies suggest that the abnormal metabolism of glycerophospholipids is associated with some important features of AD, such as neuroinflammation, neuronal injury, and neurodegeneration (60–62). Previous studies also found that several glycerophospholipids were significantly increased in the cerebrospinal fluid (CSF) samples with AD-like pathology (63, 64). Therefore, due to the importance of these brain lipids in the cognitive decline, the three combined lipid metabolites of SM (OH) C24:1, SM C24:0, and PC ae C44:3 will result in an acceptable discrimination between early-stage AD patients and MCI patients in our study.

This study has several limitations. First, the sample size was relatively small. Second, this was a cross-sectional study to compare the metabolomics between MCI and early-stage AD patients. Long-term follow-up was indicated to realize which metabolites could predict MCI or AD before the onsets, but it took many years. Our findings may serve as a pilot study. Third, there was no age-matched control group in our study. The lack of a control group was mainly due to the strict exclusion criteria of our study. Fourth, the interference of hypnotic drugs on serum metabolomics was unknown, since no published studies had discussed about the effects of different hypnotics drugs on metabolic profiles in patients with cognitive impairment. Even with these limitations, we believed the presented results might be the base of future multicentric studies, with larger samples and longer follow-up.

CONCLUSIONS

In this study, we identified the differences of serum metabolites between patients of MCI and early-stage AD. The changes of these metabolites reflect increased severity of neuronal injury and neurodegeneration in early-stage AD than MCI. The positive findings from the present serum metabolomics analyses, together with other recent findings, indicate that the serum metabolomics analysis may help to distinguish MCI patients from early-stage AD patients clinically. Long-term follow-up studies with larger samples will be performed in the future to see if these metabolites can predict the MCI progression to AD.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Review Board of Chang Gung Memorial Hospital. The patients/participants provided their written informed consent to participate in this study.
AUTHOR CONTRIBUTIONS

W-CW, W-YH, and K-HC: conceptualization. K-HC, H-YT, and M-LC: methodology. H-YT: software. K-HC, H-YT, and M-LC: validation. W-YH and H-YT: formal analysis. W-YH: data curation. W-CW and W-YH: writing—original draft preparation. K-HC and W-YH: writing—review and editing. K-HC: supervision. W-CW and W-YH: project administration. W-CW: funding acquisition. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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