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Scientific Evidence for Korean Medicine and Its Integrative Medical Research 2017

Lead Guest Editor: Wansu Park
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Editorial Board

Contents

Hindawi
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Editorial

Scientific Evidence for Korean Medicine and Its Integrative Medical Research 2017

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In 2016, the total medical fee for Korea Medicine (KM) in South Korea was over 4 billion dollars and the National Health Insurance of South Korea paid about 2.09 billion dollars for medical services provided by 19,737 KM doctors in 302 KM hospitals and 14,150 KM local clinics. Actually, 12.9 million Korean patients received 105 million outpatient services provided by KM doctors in 2016. The older the Korean society is, the more important the role of KM becomes, because Korea is anticipated to be a hyperaged society in 2028. As well as the *Donguibogam*, which was enlisted on the Memory of the Word by UNESCO in 2009, Sasang typology, Sasang Constitutional Medicine, Sa-am acupuncture, Chuna therapy, Pharmacopuncture, Korean physical therapy, Korean psychotherapy, and so on characterize KM. But more integrative researches and scientific evidences for KM might strengthen both clinical efficacy and applicability of KM. In this respect, it seems to be unavoidable that KM utilizes cutting-edge techniques of modern science. The more accurate and efficient practice of KM innovated with the modern bioscientific technology would help people live healthy lives.

Our special issue, which had opened for 6 months in the first half of 2017, focused on scientific evidence for KM and its integrative medical research.

An article by W.-W. Choi et al. described that the traumatic brain injury (TBI) mouse model was induced using the controlled cortical impact method; Chunghyul-Dan (CHD) was orally administered twice a day for 5 d after TBI induction; mice were assessed for brain damage, brain edema, blood-brain barrier (BBB) damage, motor deficits, and cognitive impairment; treatment with CHD reduced brain damage seen on histological examination and improved motor and cognitive functions; however, CHD did not reduce brain edema and BBB damage; CHD could be a candidate agent in the treatment of patients with TBI.

S. M. Hong et al. described that the aim of this study was to determine the differential effect of sleep deprivation in individuals with different body compositions (fluid) according to Soyang type (SY) and Taeeum type (TE); total body water and extracellular water were significantly different between the groups in the intervention phase; physiological parameters also varied from the beginning of the resting phase to the end of the experiment; potassium levels changed more in SY than TE individuals; participants responded differently to the same amount of sleep deprivation depending on their Sasang constitution types; this study indicated that SY individuals were more sensitive to sleep deprivation and were slower to recover from the effects of sleep deprivation than TE individuals.

J. Kim et al. described that they assessed the quality of reporting based on CAse REport (CARE) and STandards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) guideline checklists; a total of 93 eligible case reports of acupuncture treatment were identified among the 107 articles screened; overall quality of reporting in the case reports was generally acceptable (75.4% on CARE, 67.7% on STRICTA), but several crucial items remained substantially underreported; in conclusion, endorsement of the CARE and

STRICTA guidelines is needed to improve the completeness of reporting.

S.-R. Kim et al. described that they screened 189 participants aged 20 to 49 years, complaining of headache; to classify patients in terms of Yin deficiency, they used two self-reporting Yin-deficiency questionnaires and diagnosis by a doctor; based on the tests, a total of 33 subjects were assigned to a Yin-deficient group and 33 subjects were assigned to a nondeficient control group; tongue images were acquired using a computerized tongue diagnostic system, for evaluating tongue indices; in conclusion, Yin-deficient patients had less tongue coating and tended to have a more reddish tongue than nondeficient patients.

An interesting study by S. Jang et al. described that this survey aimed to investigate the characteristics of users and nonusers of herbal medicine and the adverse events experienced due to herbal medicines in South Korea; the questionnaire consisted of safety, using experience, using type, usage and nonusage reason, purchase location, and adverse events of herbal medicine; of the total 1,134 respondents, 726 (64.0%) considered herbal medicine safe, and 693 (61.1%) answered that they have taken herbal medicines within the past year; among those who took herbal medicines, 46 experienced adverse events, and the most frequently reported symptoms were digestive disorders (52.2%); regulation of herbal medicines is needed in order to resolve problems related to the safety of herbal medicines.

S. J. Jung et al. described that the distribution of mast cells (MCs) in the ventral skin of mice was studied so that it could be used to infer the locations, depths from the epidermis, and sizes of three putative acupuncture points (Aps); the harvested skins from 8-week-old mice were stained with toluidine blue, and the MCs were recognized by their red-purple stains and their metachromatic granules; the three putative APs, CV 8 and the left and the right KI 16 APs, were identified based on their high densities of MCs; these findings also imply that acupuncture may stimulate, through MCs, an immune response to allergic inflammation.

Y.-C. Yun et al. described that the Morris water maze (MWM) test demonstrated a significant improvement in hippocampal-dependent memory in the middle cerebral artery occlusion (MCAO) rats after laser acupuncture (LA) treatment; LA treatment significantly reversed the postischemic decrease in choline acetyltransferase immunoreactivity in the hippocampal CA1 region; LA treatment could improve cognitive impairment in MCAO rats to enhance the cholinergic system in the hippocampal CA1 region and to exert a neuroprotective effect by regulating Creb, Bdnf, Bcl-2, and Bax gene expressions.

J. Kim et al. described that their study aims to investigate the efficacy of Yukgunja-tang (YGJT) on functional dyspepsia (FD) patients classified by 3-dimensional facial measurement using a 3-dimensional facial shape diagnostic system (3-FSDS); a placebo-controlled, double-blind, randomized, two-center trial will be performed to evaluate the efficacy of YGJT on FD patients; the results of this trial will help the FD patients improve the symptoms and quality of life effectively and provide objective evidence for prescribing the YGJT to FD patients in clinical practice; this trial is registered with Clinical Research Information Service Identifier: KCT0001920.

S. Mun et al. described that data on cold (CP), heat (HP), spleen-qi deficiency (SQDP), and kidney deficiency (KDP) patterns were extracted by a factor analysis of symptoms experienced by 954 participants; the CP and SQDP scores were higher and the HP score was lower in women; the HP and SQDP scores decreased with age, while KDP scores increased with age; the underlying pathology of CP and SQDP might be associated with the body's metabolic rate.

In conclusion, we expect that this special issue updates scientific evidences in KM integrative research and makes a useful progress for improving KM practice.

Acknowledgments

We express our great appreciation to all authors for their excellent contributions and reviewers for their valuable help. We express our sincere thanks to the Editorial Board of eCAM for their approval on this topic and continuous support in successful publication of this special issue. The Lead Guest Editor would like to thank the three Guest Editors for their dedicated cooperation. We hope the special issue will bring readers useful academic reference in their research.

Wansu Park
Vesna Sendula-Jengic
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Research Article

Yuk-Mi-Jihwang-Tang, a Traditional Korean Multiple Herbal Formulae, Improves Hippocampal Memory on Scopolamine Injection-Induced Amnesia Model of C57BL/6 Mice

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We evaluated neuropharmacological properties of Yuk-Mi-Jihwang-Tang (YJT) against scopolamine injection-induced memory impairment mice model. Mice were orally administered with YJT (50, 100, or 200 mg/kg) or tacrine (TAC, 12.5 mg/kg) for 10 days. At the first day of Morris water maze task, scopolamine (2 mg/kg) was intraperitoneally injected before 30 min of it. The hippocampal memory function was determined by the Morris water maze task for 5 days consecutively. Scopolamine drastically increased escape latency and decreased time spent in target quadrant. Pretreatment YJT properly improved them. Regarding the redox status, YJT significantly reduced the oxidative stress and it also exerted much effort to improve both superoxide dismutase and catalase activities in hippocampal gene expression and protein levels. These effects were well coincided with immunohistochemical analysis of 4-hydroxyneal-positive signals in hippocampal areas. Additionally, acetylcholine esterase activities and brain-derived neurotrophic factor abnormalities in the hippocampal protein levels were significantly normalized by YJT, and their related molecules were also improved. The neuronal proliferation in hippocampal regions was markedly inhibited by scopolamine, whereas YJT notably recovered them. Collectively, YJT exerts much effort to enhance memorial functions through improving redox status homeostasis and partially regulates acetylcholine esterase activities as well as neuronal cell proliferation.

1. Introduction

Based on Traditional Korean Medicine (TKM) clinical practices over a thousand years, there are strong evidences of effects that various herbal medicines have on intractable diseases, especially neurodegenerative diseases [1–3]. Regarding the neurodegenerative diseases, Alzheimer's disease and Parkinson's disease have become a critical medical issue in the world recently, particularly aging population [4–6]. According to the previous study, approximately around 34 million people have suffered from Alzheimer's disease, and among them 5 ≥ million people from the United States have been diagnosed as Alzheimer's disease patients [7].

Additionally, the pathophysiological features of neurodegenerative diseases are clinically progressed and developed by loss of cognitive abilities, which affects learning and memory dysfunction [8, 9]. Deposition of amyloid plaques, tau protein aggregation, cerebral oxidative stress, neuroinflammation,

Table 1: Components of *Yuk-Mi-Jihwang-Tang* and its ratio.

Herbal name	Specific name	Used amount (g)
Rehmanniae Radix Preparata	*Rehmannia glutinosa* Liboschitz *var. purpurea* Makino	200
Dioscoreae Rhizoma	*Dioscorea japonica* Thunb.	100
Corni Fructus	*Cornus officinalis* Sieb. et Zucc.	100
Moutan Cortex Radicis	*Paeonia moutan* Sims.	75
Alpiniae oxyphyllae Fructus	*Alpinia oxyphylla* Miq.	75
Schizandrae Fructus	*Schisandra chinensis* Baill.	50
Total amount	-	600

and cholinergic dysfunction were mainly accompanied with neurodegenerative diseases, and they can lead to psychological and pathophysiological complications such as anxiety, depression, concentration problems, and motor disturbances [10, 11]. Among the various symptoms of them, the memory impairment is mainly provoked by cholinergic system abnormality that involves cholinergic neurons, neurotransmitters, and their receptors [12]. The etiology and pathogenesis of neurodegenerative disorder, however, remain unclear till recent days. Among the various regions of brain tissue, only some parts revealed that the cholinergic dysfunctions are attributed to the loss of cholinergic neurons in the basal fore brain and hippocampus impairs cognitive ability [13, 14].

In the normal status, the cholinergic activity in the central nervous system (CNS) leads to contribution of hippocampal neuronal genesis and memory improvement through the cAMP response element-binding protein/brain-derived neurotrophic factor (CREB/BDNF) signaling pathways [15]. According to above reasons, the primary accessible treatment in clinic used the modulations of acetylcholinesterase (AChE) inhibitors such as tacrine or donepezil, which increase the availability of acetylcholine at cholinergic synapses [16]. This drug, which is thought to be a potent treatment on the neurodegeneration related therapeutics, however, is still needed to prove its efficacy clinically.

Contrary to western medicine, the TKM has recognized that neurodegenerative diseases were frequently aroused due to an imbalance of qi and blood flows, which are main factors of the human body. Among the various herbal medicines, *Yuk-Mi-Jihwang-Tang* (YJT), which is well known to a representative Korean Traditional herbal medicine, has been popularly used for patients with various disorders including aging-related disorders, obesity, ischemia, and immune suppression for hundreds of years in South Korea [17–19]. Particularly, the YJT showed its pharmacological properties on the aging-related diseases, especially enhancement of memorial function evidenced by scientific experiments until recent days [20]. However, there is no study about its therapeutic efficacies against AChE inhibitors of memory deficits model.

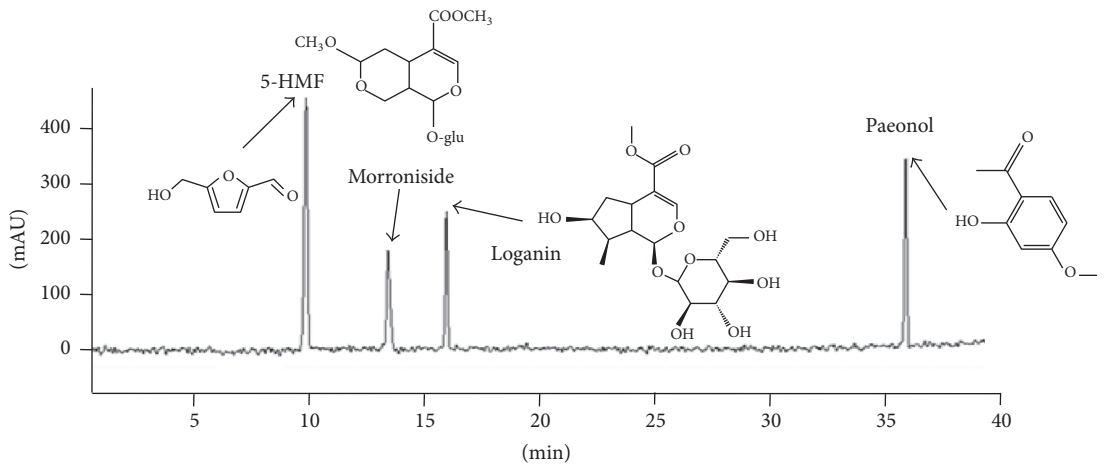
Thus, in the present study we investigated the antiamnesic effects of YJT on memory deficits in a mouse model of cognitive impairment by Ach system abnormality which is induced by single injection of scopolamine using mice model.

2. Materials and Methods

2.1. Preparation of YJT. YJT comprises 6 kinds of herbs including *Prepared Rehmannia glutinosa* Liboschitz *var. purpurea* Makino, *Dioscorea japonica* Thunb., *Cornus officinalis* Sieb. et Zucc., *Paeonia moutan* Sims, *Alpinia oxyphylla* Miq., and *Schisandra chinensis* Baill.; all these herbs were mixed with differential ratio of 4 : 2 : 2 : 1.5 : 1.5 : 1 (Table 1). All the herbal plants were obtained from the Dunsan Oriental Hospital of Daejeon University with inspection of Herbology professor (Daejeon, Republic of Korea). The herbal mixtures (total weights were 600 g) were boiled with distilled water (DW) at 100°C for 4 h and then filtered using a 300 mesh filter (50 μm). After condensing the extract for 1 h, it was placed under −70°C for at least 3 h. The frozen extract was processed to the frozen lyophilization for 72 h and collected them and weighed. The final yield was 15.51% (w/w).

2.2. Fingerprinting Analysis of YJT. To verify the identification of used herbs and reproducibility of YJT preparation, the fingerprinting was performed using high-performance liquid chromatography-diode array detector-mass spectrometry (HPLC-DAD-MS) for YJT and its four reference compounds as follows: *Rehmannia glutinosa* Liboschitz *var. purpurae* Makino versus 5-hydroxymethyl-2-furfural (5-HMF), *Cornus officinalis* Sieb. et Zucc versus loganin and morroniside, and *Paeonia moutan* Sims versus paeonol, which were followed by the our previous conditions [21]. Briefly, after the dissolution (20 mg of YJT and 2 mg of each of the six herbal extracts in 1 mL of water; 0.01 mg of 8 standards in 1 mL of water or 50% methanol) and filtration, these formulations were subjected to HPLC analysis of Agilent 1100 series (Agilent Technologies, Santa Clara, CA). The HPLC system consisted of a SCL-10A system controller, LC-10AD pump, SPD-10MVP diode array detector, and CTO-10AS column temperature controller (Shimadzu, Kyoto, Japan). A Phenomenex Prodigy C18 (4.6 × 250 mm; particle size 5 μm; Phenomenex, Torrance, CA) column was eluted with solvents A (10% acetonitrile in water containing 0.1% formic acid) and B (DW) at a flow rate of 0.4 mL/min. Solutions of 15% A and 85% B were changed to 60% B for 30 min, 40% B for 40 min, and 0% B for 60 min (Figures 1(a)–1(c)).

2.3. Animals and Experiment Plan. A total seventy of specific pathogen-free C57BL/6J male mice (12 weeks old, 24–26 g)



Contents	5-HMF	Morroniside	Loganin	Paeonol
RT (min)	9.64	13.37	16.00	36.78
Con. (ug/mg)	0.49 ± 0.03	6.16 ± 0.05	1.70 ± 0.02	0.08 ± 0.01

(c)

Figure 1: *Fingerprinting analysis of YJT and its reference compounds.* For fingerprinting analysis for YJT, YJT and its major reference compounds were subjected to the high-performance liquid chromatography (HPLC). Two-dimension histogram of YJT (a) and its four major compounds (b). Quantitative analysis of five reference components in YJT (c).

were procured from Orient Bio (Gyeonggi-do, South Korea). The mice had free access to food pellets (Orient Bio, Gyeonggi-do, Korea) and water ad libitum and were kept in an animal room (temperature at 23 ± 2°C and humidity for 60 ± 5%, with a 12 h:12 h light and dark cycle). After 7 days of acclimatization, the mice were randomly divided into six groups ($n = 10$ for normal and $n = 12$ for other of each treatment groups): normal (no restraint stress with DW), Sco. Only group (scopolamine injection with DW), YJT groups (scopolamine injection with 50, 100, or 200 mg/kg), and Tac. group (scopolamine injection with tacrine 12.5 mg/kg). The YJT and tacrine were dissolved in DW and orally administered using dosing gavage for 7 consecutive days. On the 7th day of the experiment, all the mice were intraperitoneally injected to neutral saline (0.9%) or scopolamine (2 mg/kg), 1 h prior to Morris waster maze task at the first day. The Morris waster maze task was complete after further 4 consecutive days.

All the animal experiment procedures were approved by the Korea Food Research Institute (Gyeonggi-do, South Korea). The animal experiment was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH).

2.4. Morris Water Maze Task. The Morris water maze task was performed according to the previous method [22]. Briefly, a circular pool (100 cm diameter 3 × 50 cm height) with a circular acrylic platform (10 cm diameter 3 × 35 cm height) was used and location of platform can be discriminated by visual cue. The pool was filled with milk water (22 ± 1°C) and divided into equal quadrants [23]. Data were

recorded using a video camera connected to the corresponding software (Smart Junior, Panlab SL; Barcelona, Spain). Mice were placed on the platform for 10 s and removed from the pool. Mice were given acquisition trial for 4 days. The escape latency and cumulative path-length were recorded during each acquisition trial. On day 5th, all mice were subjected to probe trial without platform and were recorded for 120 s. The time spent in the target quadrant was measured for spatial learning and memory.

2.5. Preparation of Brain Tissue and Serum Samples. All mice were sacrificed under ether anesthesia condition after 1 h following the Morris water maze. Whole blood samples were isolated via abdominal vein and serum was collected by centrifugation at 3000 rpm for 15 min at 4°C. The hippocampal region was isolated from the whole brain tissue immediately, and then samples were stored at −80°C or in RNAlater (Ambion, TX). In each group, two mouse brains tissues were fixed in 4% paraformaldehyde (PFA) for immunohistochemistry (IHC) analysis after cardiac perfusion. Hippocampal areas in each hemisphere of remaining eight to ten mice were used for other experiment including biochemical analysis, western blot, and real-time PCR analysis. The part of hippocampus was homogenized on ice using radio-immunoprecipitation assay (RIPA) buffer and other parts of hippocampus were used for isolation of RNA.

2.6. Assessments of Laboratory Assays. For the determination of oxidative stress parameters and antioxidant component assays in the hippocampal area, the part of stored hippocampal tissue homogenates was used (n = 8 to 10 in each group). All assays were measured by commercial kit.

The lipid peroxidation was determined by the commercial product of Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit (Catalog#, K739, Bio Vision, Milpitas, CA). The final products of MDA were measured at 530 nm using a spectrophotometer (Soft Max, Ver. 5.4, Molecular Devices, Sunnyvale, CA). Total glutathione (GSH) content was determined using a commercial kit (OxiSelect™, Catalog# STA-312, Cell Bio Labs, INC. San Diego, CA) with the absorbance measured at 405 nm using a spectrophotometer (Soft Max, Ver. 5.4, Molecular Devices). SOD activities were determined using an SOD assay kit (Dojindo Laboratories, Kumamoto, Japan), and dilutions of bovine erythrocyte SOD (St. Louis, MO) ranging in concentration from 0.01 to 50 U/ml were used as standards. Catalase activities were determined using a commercial kit (OxiSelect™, Catalog# STA-314, Cell Bio Labs, INC.). All procedures were carried out, according to the manufacturer's protocol. The absorbance was measured at 450 nm using a spectrophotometer (Soft Max, Ver. 5.4, Molecular Devices).

2.7. Western Blot Analysis. Each hippocampal area from whole brain tissues was homogenized in RIPA buffer with proteinase inhibitor solution for performance of western bloat analysis (n = 8 to 10 in each group). Protein samples (concentration was about 30 μg to each sample) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA). The membranes were then incubated in PBS containing 5% nonfat powdered milk and 0.1% Tween 20 for 1 h to block using nonspecific binding before being incubated with primary antibodies for 4-HNE (#ab46545, Abcam, Cambridge, MA), iNOS (sc-7271, Santa Cruz Biotechnology Dallas, TX), and β-actin (sc-1616, Santa Cruz Biotechnology, Dallas, TX) overnight at 4°C in blocking solution (5% skimmed milk in 0.1% Tween 20 in 10 mM PBS, pH 7.3). The blots were washed and incubated with HRP-conjugated secondary antibody for 1 h at room temperature, and the peroxidase activity was detected using the Immobilon Western HRP detection reagent (Millipore) using an Image Reader (Thermo Fisher Scientific, Rockford, IL). The β-actin was used as a reference protein for all the results. The ratio of the protein of interest was subjected to β-actin.

2.8. Gene Expression Analysis. For analysis of the expressions of eight genes, the total RNA was isolated from hippocampal area (n = 4 to 5 in each group) using an RNeasy Mini Kit (QIAGEN, Valencia, CA). The cDNA was synthesized by using a cDNA synthesis kit (Invitrogen, Waltham, MA). The real-time PCR was performed using SYBR Green PCR Master Mix (Roche), and the PCR amplification was performed using a standard protocol with the IQ5 PCR Thermal Cycler (Bio-Rad, Hercules, CA). The detected genes for the gene expression analysis include the following: brain-derived GSH syntheses (GSS), GSH-reductase (Grd), GSH-peroxidase (Gpx), SOD-1, SOD-2, and SOD-3, muscarinic Ach receptor 1 (mAchR1), cAMP response element-binding protein (CREB), CREB-binding protein (CBP), and brain-derived neurotrophic factor (BDNF) were measured by real-time PCR. The primer sequences, product sizes, and annealing temperatures are summarized in Table 2. For analyzing the mRNA expression results using calculated the fold changes, the GAPDH was used as a reference gene.

2.9. Determination of AChE Activities and Brain-Derived Neurotrophic Factor (BDNF) Contents in Hippocampal Area. Acetylcholinesterase (AChE) activities and BDNF contents were measured in hippocampus using commercial colorimetric AChE activity assay kit (#ab138871, Abcam, CA, and #ab212166, Abcam) according to the manufacturer's protocol.

2.10. Determinations of IHC Analysis in the Brain Tissue. In other measurements of neuronal cell proliferation in the hippocampal area, the Ki67 staining was performed, and for determining lipid peroxidation, a final product of oxidative stress, the 4-HNE staining was also performed. At the final day of experiment, two mice in each group were progressed by cardiac perfusion using 4% PFA solution until blood was appeared clearly (200 to 300 mL of solution). After perfusion process, brain tissues were isolated and cryoprotected in the state of gradient sucrose solutions (10, 20, and 30%) for 24 h, embedded in tissue-freezing medium with liquid nitrogen, and cut into coronal frozen sections (40 μm) using a cryostat.

Table 2: Sequence of the primers used in real-time PCR analysis.

Gene list (NCBI number)	Primer sequencing (forward and reverse)	Product size (base pair)	Annealing temperature (°C)
GSS (NM-001291111)	5′-ACC GAA GGC TGT TTA TGG ATG A-3′ 5′-AGG CGT GCT TCC CAG TTC T-3′	100	59
Grd (NM-010344.4)	5′- GAT GTG TGG AGC GGT AAA CTT TT -3′ 5′- AGC CGC CRG AAC ACC ATC TA -3′	120	60
Gpx (NM-001329527.1)	5′-CTC ACC ATT CAC TTC GCA CTT C-3′ 5′-ACA CCA GGA GAA TGG CAA GAA-3′	122	59
SOD-1 (NM_011434)	5′-TGT CAG GAC AAA TTA CAG GAT TAA CTG-3′ 5′-AAA TGA GGT CCT GCA CTG GTA CA-3′	100	60
SOD-2 (NM_013671)	5′-CCCAGACCTGCCTTACGACTAT-3′ 5′-GGTGGCGTTGAGATTGTTCA-3′	112	58
SOD-3 (NM_011435)	5′-GGT GGA TGC TGC CGA GAT-3′ 5′-GCT GCC GGA AGA GAA CCA A-3′	101	59
SOD-3 (NM-011435)	5′-GGT GGA TGC TGC CGA GAT-3′ 5′-GCT GCC GGA AGA GAA CCA A-3′	101	59
mAChR 1 (NM-001112697)	5′-AGT GGC ATT CAT CGG GAT CA-3′ 5′-CTT GAG CTC TGT GTT GAC CTT GA-3′	100	60
CBP (NM-001025432)	5′-CTG GCA GAC CTC GGA AAG AA-3′ 5′-CTG GCG CCG CAA AAA CT-3′	100	59
CREB 1 (NM-013497)	5′-ACA GTG CCA ACC CCC ATT TA-3′ 5′-GTA CCC CAT CCG TAC CAT TGT T-3′	100	59
BDNF (NM-001048139)	5′-CAC TTT TGA GCA CGT CAT CGA A-3′ 5′-CAC CCG GGA AGT GTA CAA GTC-3′	104	60
GAPDH NM_001289726	5′-TCA CTC AAG ATT GTC AGC AAT GC-3′ 5′-GGC CCC GGC CTT CTC-3′	100	58

BDNF: brain-derived neurotrophic factor, CREBP: cAMP response element-binding protein, CBP: CREBP binding protein, GSS: glutathione synthase, Grd: glutathione-reductase, Gpx: glutathione-peroxidase, mAchRI: muscarinic acetylcholine receptor 1, SOD: superoxide dismutase.

Cryosections of each brain tissue were moved in to microscopy slide and were subjected to endogenous peroxidase quenched with 3% H_2O_2 in PBS (pH 7.3). And then tissues were treated by blocking buffer (5% normal chicken and goat serum in PBS for overnight at room temperature, RT), incubated with primary antibodies using 4-HNE (1 : 125, #ab48506, Abcam), and then washed using PBS (pH 7.3). After discarding the primary antibody, tissues were further incubated with a biotinylated goat anti-rabbit secondary antibody (1 : 250, #ab64256, Abcam). The tissues were exposed to an avidin-biotin peroxidase complex (Vectastain ABC kit, Vector) for 2 h. The peroxidase activity was visualized using a stable diaminobenzidine solution. Immunoreactions were observed using a microscope circumstance under the magnification of ×200 (Olympus, Germany) and quantified using the Image J 1.46 software (NIH, Bethesda, MD).

2.11. Statistical Analysis. All obtained data were expressed as mean ± standard deviation (SD). Statistically significant differences between the groups were analyzed by one-way analysis of variance (ANOVA) followed by post hoc multiple comparisons with Bonferroni t-test using the IBM SPSS statistics 20.0 (SPSS Inc., Chicago, IL). Differences at $p < 0.05$ were considered statistically significant.

3. Results

3.1. Fingerprinting Analysis of YJT. To obtain the chemical composition of the YJT, we subjected it to the HPLC equipment following the same conditions of our previous study [21]. We finally detected a total 4 of the reference compounds from YJT. Firstly, we identified 5-HMF which is a reference compound of *Rehmannia glutinosa* Liboschitz *var. purpurae* Makino at 9.46 min of retention time and its concentration was 0.49 ± 0.03 μg/mg. Two of the compounds, such as loganin and morroniside, were detected from *Cornus officinalis* Sieb. et Zucc. at the retention time of 13.37 and 16.00 min, and the concentration was 6.16 ± 0.05 μg/mg and 1.70 ± 0.02 μg/mg, respectively. The paeonol was detected from *Paeonia moutan* Sims. in 36.78 min, and its concentration was 0.08 ± 0.01 μg/mg (Figures 1(a)–1(c)).

3.2. Effects of YJT on the Memorial Dysfunction Analysis Using Morris Water Maze Task. To estimate the pharmacological effects of YJT on the memorial dysfunction, we performed the Morris water maze test for 4 consecutive days for adaptable trials. At that day of 5th, scopolamine injection significantly caused to increase the escape latency, and the time spent in the target quadrant was significantly reduced by single

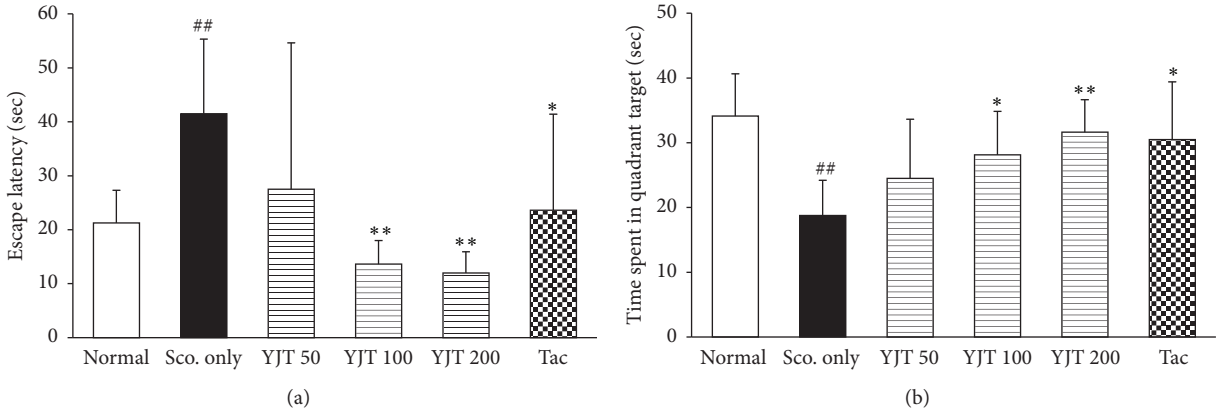


Figure 2: *Effects of YJT on the spatial learning and memory functional analysis using the Morris water maze.* The escape latency (a) and time spent in the target quadrant (b). Data are expressed as the mean ± SD (n = 10 to 12). $p^{\#\#} < 0.01$ versus normal group; $p^{*} < 0.05$ and $p^{**} < 0.01$ versus scopolamine only injection group.

injection of scopolamine as compared with normal group ($p < 0.01$ for both parameters, Figures 2(a) and 2(b)). On the other hand, preadministration with YJT (100 and 200 mg/kg) significantly improved both the escape latency ($p < 0.01$) and time spent in the target quadrant compared with the scopolamine only group ($p < 0.05$ or $p < 0.01$, Figures 2(a) and 2(b)). Preadministration with tacrine (12.5 mg/kg), used as positive drug in the current study, displayed similar effects of YJT.

3.3. Effects of YJT on the Hippocampal Protein Levels of GSH Contents, MDA, and Activities of Catalase and SOD. Regarding oxidative stress and antioxidant components, scopolamine aggravates the redox status imbalance in hippocampus especially. Thus, we determined the redox status by determinations of GSH contents, MDA, and activities of catalase and SOD, respectively. Hippocampal protein level of total GSH was decreased as 32%, but MDA was increased around 88% as compared with normal group. Those abnormal alterations by scopolamine injection were significantly normalized by preadministration with YJT compared to the scopolamine only group ($p < 0.05$ in 200 mg/kg, Figures 3(a) and 3(b)). These effects were well coincided with catalase activities in hippocampal area. The catalase activities were considerably decreased as 68% of normal group, whereas by preadministration with YJT ($p < 0.05$ for 200 mg/kg, Figure 3(c)), the SOD activities were not influenced by both scopolamine injection and YJT (Figure 3(d)).

Tacrine also considerably exerted to reduce the oxidative stress and ameliorate antioxidant components, but not SOD activities (Figures 3(a)–3(d)).

3.4. Effects of YJT on the Hippocampal Regions of 4-HNE and iNOS in Protein Levels and Gene Expression Levels of Antioxidant Components. To verify the antioxidant effects of YJT against scopolamine-induced hippocampal injury in the current model, we further examined IHC against to the 4-HNE antibodies. As we expected the positive signals (shown as deep brown color) in hippocampal areas were notably enhanced by scopolamine injection, and preadministration with YJT (particularly 200 mg/kg) significantly ameliorated those abnormalities (Figure 4(a)). Western blot analysis also well supported the pharmacological properties of antioxidant. The iNOS and 4-HNE in the hippocampal protein levels were markedly increased after being faced with scopolamine injection approximately 3.0- and 4.0- fold and theses oxidative injuries compared to normal group. Preadministration with YJT, however, significantly reduced those abnormal augmented of oxidative stress injuries as normal levels ($p < 0.05$ for 200 mg/kg, Figures 4(b)–4(d)).

Next, we performed the real-time PCR to investigate the degrees of antioxidant abilities in the gene expression level in the hippocampal regions. The GSS, Grd, Gpx, SOD-1, SOD-2, and SOD-3 were significantly lowered than that of normal group approximately 0.75-, 0.62-, 0.71-, 0.65-, 0.75, and 0.9-fold, respectively, as compared with normal group. However, pretreatment with YJT significantly normalized the above alterations as a normal level ($p < 0.05$, Figure 4(e)).

The similarities of YJT were also occurred by preadministration with tacrine in IHC against to 4-HNE signals and western blot analysis ($p < 0.05$, Figures 4(a)–4(d)), and tacrine also led to normalization of Grd, Gpx, and SOD-1 gene expression levels ($p < 0.05$, Figure 4(e)).

3.5. Effects of YJT on AChE Activities and BDNF Contents. To investigate the possible mechanisms of YJT against the scopolamine injection-induced amnesia, we measured the hippocampal protein levels of AChE activity and BDNF. In the present study, we confirmed that the scopolamine considerably caused approximately 3.5-fold increases of AChE activities compared to the normal group. Preadministration with YJT (200 mg/kg), however, significantly ameliorated the abnormal elevations of AChE activities in hippocampal regions ($p < 0.05$, Figure 5(a)).

The BDNF, which is known for improving both the learning abilities and neurogenesis, in the hippocampal region contents was markedly lowered as 0.4-fold compared to that of normal group, whereas preadministration with

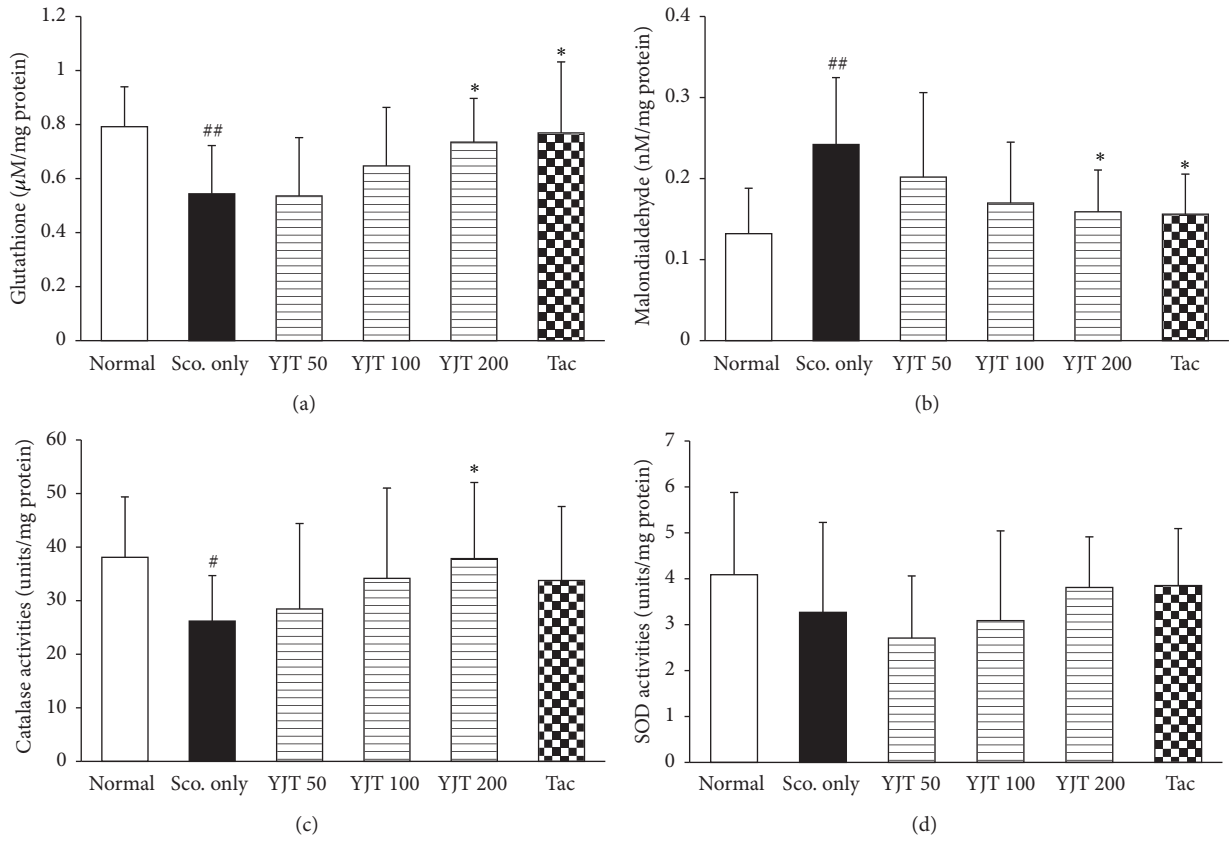


Figure 3: *Effects of YJT on the hippocampal protein levels of oxidative stress and antioxidant-related molecules.* Hippocampal tissue protein levels of glutathione (a), malondialdehyde (b), catalase activities (c), and SOD activities (d). Data are expressed as the mean ± SD (n = 8 to 10). $p^{\#} < 0.05$ and $p^{\#\#} < 0.01$ versus normal group; $p^{*} < 0.05$ versus scopolamine only injection group. SOD: superoxide dismutase.

YJT significantly recovered as normal levels compared to the scopolamine only injection group ($p < 0.05$, Figure 5(b)).

Tacrine group had similar effects of YJT in the present study (Figures 5(a) and 5(b)).

3.6. Effects of YJT on Neuronal Cell Proliferation Related Molecules. To verify the pharmacological mechanism of YJT properties on the memory function enhancement, we further examined the gene expression analysis of memorial function related molecules including mAchR1, CBP, CREB1, and BDNF and estimated the neuronal cell proliferation for IHC against Ki67 antibody.

The gene expression levels of mAchR1, CBP, CREB1, and BDNF were considerably downregulated in scopolamine only injection group as 0.61-, 0.71-, 0.5-, and 0.5-fold compared to normal group, whereas these abnormal alterations were significantly normalized as normal level by preadministration with YJT as normal levels ($p < 0.05$ or $p < 0.01$ in YJT 100 or 200 mg/kg, Figure 5(c)).

Regarding the neuronal cell proliferation, the positive cells of the positive cells of Ki67 staining were significantly decreased approximately 0.7-fold due to scopolamine injection group as compared with the normal group, whereas preadministration with YJT (200 mg/kg) significantly recovered those of Ki67 positive cells in dentate gyrus as compared with the scopolamine only injection group ($p < 0.01$, Figures 5(d) and 5(e)).

The preadministration with tacrine also showed similar effects on the gene expression levels of mAchR1, CBP, and BDNF, and Ki67, respectively ($p < 0.05$ or $p < 0.01$, Figures 5(c)–5(e)).

4. Discussion

In the current study, we answered our questions and proved them based on the scientific experiments whether the YJT efficiently protected a single scopolamine injection-induced memory deficits using C57/BL 6 mice model. Furthermore, we proved the pharmacological properties of YJT regarding on the Ach system modulated signaling pathways mainly.

First of all, we observed the therapeutic effects of YJT on the memory deficit, which is induced by scopolamine injection, using Morris water maze test. As our expectation, YJT significantly ameliorated the memory deficit as evidence by improvement of both the time spent of quadrant target and escape latency (Figures 2(a) and 2(b)). As a key organ of memorial reorganization and its functions, next we further explored to prove the possible pharmacological mechanisms of YJT.

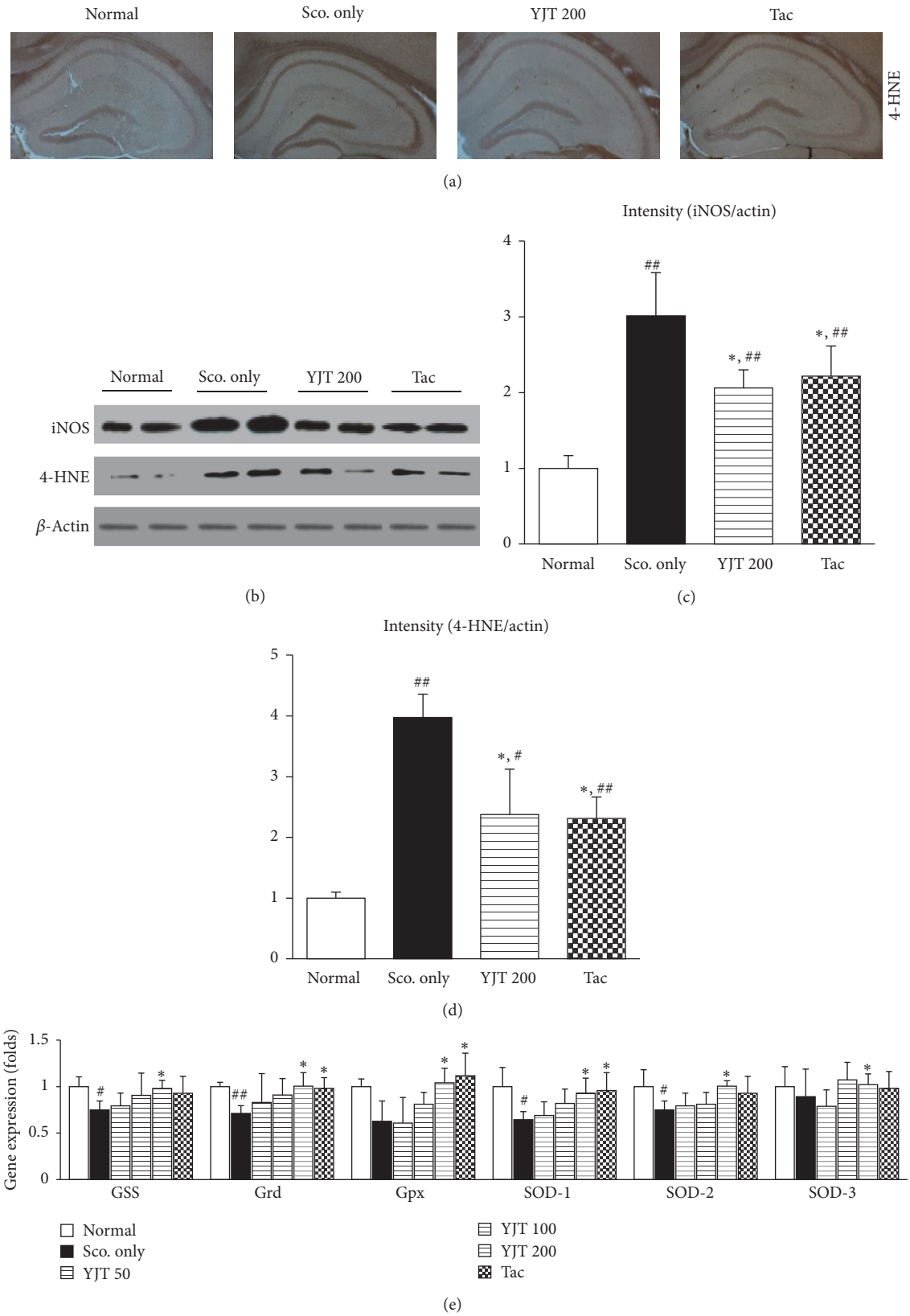


Figure 4: *Immunohistochemistry, western blot analysis, and gene expression analysis of redox status in the hippocampal area.* Immunohistochemistry analysis against the 4-HNE (a). Western blot analysis of iNOS and 4-HNE in the hippocampal regions (b) and their protein intensities (c, d). Gene expression analysis of antioxidant-related molecules including GSS, Grd, Gpx, SOD-1, SOD-2, and SOD-3 (e). Data are expressed as the mean ± SD ($n = 2$ for ICH analysis, $n = 8$ to 10 for western blot analysis, and $n = 4$ to 5 for gene expression analysis). $p^{\#} < 0.05$ and $p^{\#\#} < 0.01$ versus normal group; $p^{*} < 0.05$ versus scopolamine only injection group. 4-HNE: 4-hydroxyneal, GSS: glutathione-synthase, Grd: glutathione-reductase, Gpx: glutathione-peroxidase, and SOD: superoxide dismutase.

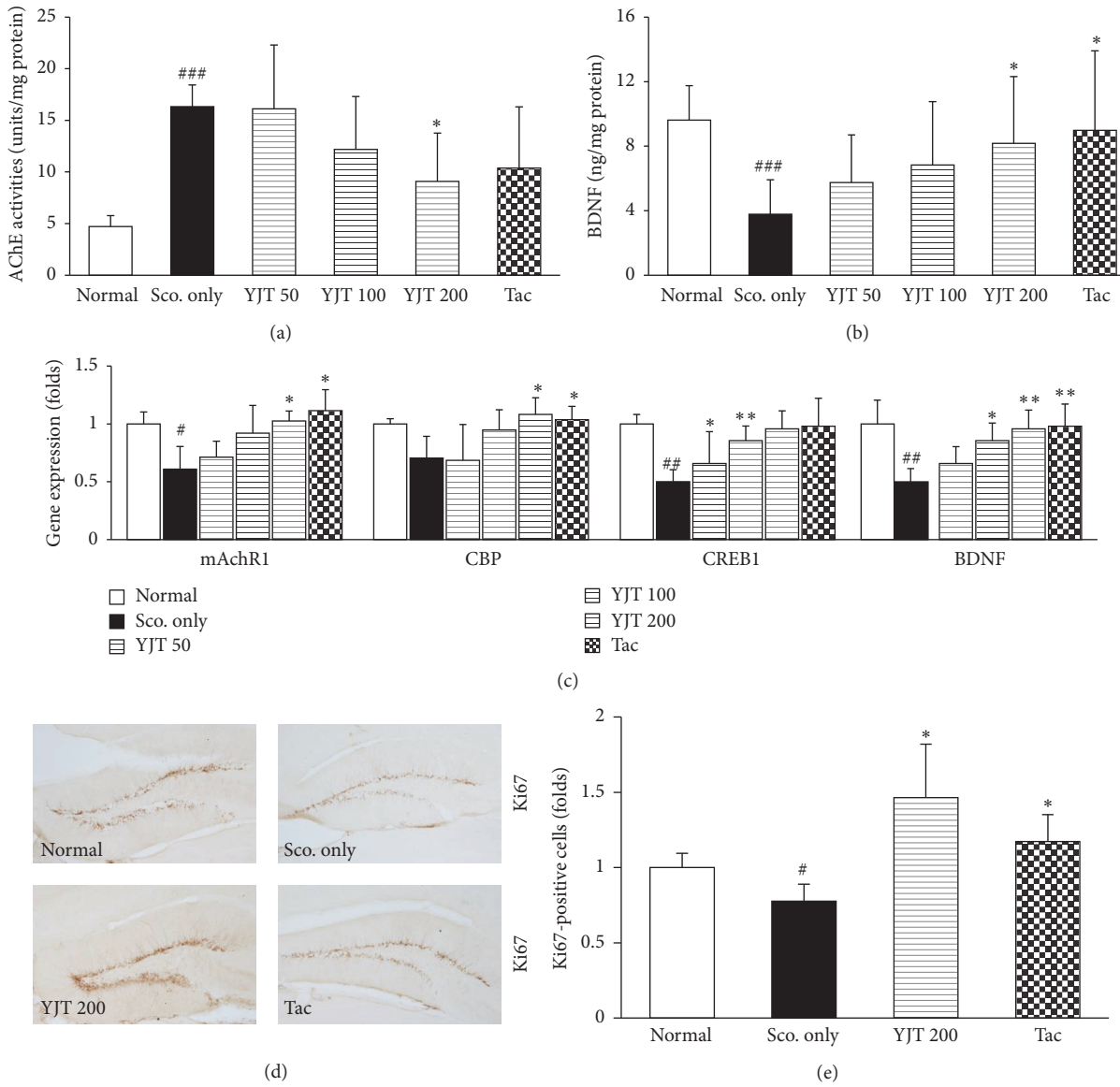


Figure 5: *Biomolecular analysis of memorial dysfunction and learning enhancement.* AChE activities analysis (a), BDNF contents (b), gene expression analysis of mAchR1, CBP, CREB1, and BDNF (c). Analysis of IHC against Ki67 (d) and its immunoreaction density (e). Data are expressed as the mean ± SD (n = 8 to 10 for AChE activities analysis and BDNF concentration; n = 4 to 5 for gene expression analysis; n = 2 for ICH analysis, resp.). $p^{\#} < 0.05$, $p^{\#\#} < 0.01$, and $p^{\#\#\#} < 0.001$ versus normal group and $p^{*} < 0.05$ and $p^{**} < 0.01$ versus scopolamine only injection group. AChE: acetylcholinesterase, mAchR1: muscarinic acetylcholine receptor 1, BDNF: brain-derived neurotrophic factor, CREBP: cAMP response element-binding protein, and CBP: CREBP binding protein.

The major possible pathophysiological mechanisms of neurodegeneration diseases are deeply linked to the central cholinergic system alterations, including cholinergic neurons, neurotransmitters, and their receptors [9, 14]. Thus, the therapeutics access is mainly focused on the inhibition of AChE and N-methyl-D-aspartate (NMDA) receptors for treating Alzheimer's disease currently [24, 25]. For proving them, we next measured the AChE activities in the hippocampal area in the brain tissue. As our expectation, the scopolamine injection considerably elevated AChE activities, whereas YJT efficiently block it (Figure 5(a)). Besides, the AChE activity is also closely linked to the cyclic adenosine monophosphate/protein kinase A- (cAMP/PKA-) CREB signaling pathway via G-coupled protein receptors [26]. Under the condition of excessive AChE activities by itself, this also can lead to provoking ACh disruption especially in hippocampal cholinergic synapses [27]. Moreover, the gene expression levels of mAChR-138 were considerably depleted to the patients with Alzheimer's disease that is well coincided with memory disruption in a mAChR-1 knockout mouse model [28, 29]. In the current study, we partially proved the YJT effects above them in the hippocampal area by performance on the gene expression analysis (Figure 5(c)). In our study, YJT also efficiently improved the deterioration of

BDNF in protein and gene expression levels of hippocampal tissues (Figures 5(b) and 5(c)).

On the other hand, CREB, which is well known to the transcription factor of BDNF, is essential molecule for working of memory and synaptic plasticity in the CNS. If phosphorylated-CREB in the hippocampal region is disrupted, it can augment neurodegenerative diseases [30]. Furthermore, previous studies well showed that the CREB play roles as neuroprotective properties on the oxidative stress-induced neuronal damage model [31]. Thus, CREB activation is most critical issue to ameliorate cognitive impairment on the therapeutic access of neurodegenerative diseases [32]. In the current study, we partially proved the YJT effects on the above them in the hippocampal area by performance on the gene expression analysis (Figure 5(c)). In our study, YJT also efficiently improved the deterioration of BDNF in protein and gene expression levels of hippocampal tissues (Figures 5(b) and 5(c)). Regarding the enhancement of learning ability and neurogenesis, BDNF directly leads to improve them via phospho-CREB signaling pathways. The IHC staining against the Ki67 well supported the above results (Figure 5(d)).

Additionally, brain tissue is well known to susceptibility to the oxidative damage, and previous reports well evidenced the relevance between oxidative stress and neurodegenerative diseases. Thus, we further examined the antioxidant effects of YJT scopolamine injection-induced neurodegenerative model in the current study. The total GSH content in the hippocampal area was significantly recovered by YJT treatments, and it is also notably exerted to decrease MDA levels, which is a final product of lipid peroxidation (Figures 3(a) and 3(b)). The GSH is a predominant antioxidant peptide that directly quenches to oxidize-protein or peptide adducts especially oxidative stress damage condition. This potent antioxidant plays a pivotal role for maintaining homeostasis between oxidative stress and antioxidant. Regarding the enzymatic-antioxidant we observed that the catalase activities were significantly increased by YJT treatments (Figure 3(c)), but not SOD. Interestingly, both the scopolamine and YJT treatments did not affect the SOD levels in the hippocampal areas (Figure 4(d)). Evidenced by numerous of previous studies, brain tissue generally consumes high rate of oxygen, has relatively low amount of antioxidant components [33], and contains abundant amount of polyunsaturated fatty acids [34], transition metal irons, and high sensitivity of blood brain endothelial cells [35]. The above previous studies are well comprised of the antioxidant properties of YJT.

In the TKM theory, which has been practiced for thousand years, the main reasons of the excess stress are evoked from the imbalance between *"Qi"* and *"blood"* streams, the two of essential components of the human body. Based on the above theory, the YJT has been popularly prescribed for treating patients with neurodegenerative diseases such as memory deficit, mild senile dementia, anxiety, or depression over than hundreds of years in clinical practice [36]. Besides, previous studies also are well reflected regarding antiamnesic effects of the YJT. The potent components of herbal plants from the YJT such as Alpiniae oxyphyllae Fructus *(Alpinia oxyphylla Miq)* and Schizandrae Fructus *(Schisandra chinensis Baillon)* showed pharmacological effects on the brain damage using animal based *in vivo* models [37–39].

Although we firstly proved the pharmacological properties of YJT on the scopolamine-induced dementia mice model, the limitation remained. In the current study, we used the scopolamine to induce neurodegenerative disease-evoked memory deficit condition, but this drug can only partially mimic some extent of memory impairments such as mild-to-moderate Alzheimer's disease due to cholinergic system disruption [40]. It can not entirely represent the senile plaque and tau accumulations, which are hallmarks of Alzheimer's dementia. Therefore, we need to investigate the neuroprotective effects of YJT using another potent animal model such as in near future [41]. Next, we still unclearly know what kind of component in YJT could be positively worked to the antineurodegenerative effects. Thus, further study will be needed to figure out which individual herbal medicine or its derived chemical components will be effective or not based on the fingerprinting analysis data.

5. Conclusion

In the current study, we investigated the antioxidant properties of the YJT under conditions of single injection of scopolamine-induced brain memory deficit of C57/BL6 mice model which can mimic the pathological condition of neurodegenerative diseases. The possible mechanisms of the YJT were improvement of antioxidant components as well as enhancement of Ach activity signaling pathways. The further, however, study will be needed to examine the effects of YJT in the clinical trial.

Conflicts of Interest

All authors declared that there are no conflicts of interest.

Authors' Contributions

Ho-Ryong Yoo and Hyeong-Geug Kim equally contributed to this work.

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References

[1] Y.-S. Ho, K.-F. So, and R. C. Chang, "Drug discovery from Chinese medicine against neurodegeneration in Alzheimer's and vascular dementia," *Chinese Medicine*, vol. 6, article 15, 2011.

[2] Z.-K. Sun, H.-Q. Yang, and S.-D. Chen, "Traditional Chinese medicine: a promising candidate for the treatment of Alzheimer's disease," *Translational Neurodegeneration*, vol. 2, no. 1, article 6, 2013.

[3] C. F. Wu, W. Zhou, C. Suthisisang, and J. Y. Yang, "Traditional medicine for treatment of neurodegenerative diseases,"

Evidence-Based Complementary and Alternative Medicine, vol. 2014, Article ID 169821, 2014.

[4] J. T. Pedersen and N. H. H. Heegaard, "Analysis of protein aggregation in neurodegenerative disease," *Analytical Chemistry*, vol. 85, no. 9, pp. 4215–4227, 2013.

[5] C. A. Ross and M. A. Poirier, "Protein aggregation and neurodegenerative disease," *Nature Medicine*, vol. 10, pp. S10–S17, 2004.

[6] B. Wolozin, "Physiological protein aggregation run amuck: Stress granules and the genesis of neurodegenerative disease," *Discovery Medicine*, vol. 17, no. 91, pp. 47–52, 2014.

[7] D. E. Barnes and K. Yaffe, "The projected effect of risk factor reduction on Alzheimer's disease prevalence," *The Lancet Neurology*, vol. 10, no. 9, pp. 819–828, 2011.

[8] M. S. Albert, S. T. DeKosky, D. Dickson et al., "The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease," *Alzheimer's & Dementia*, vol. 7, no. 3, pp. 270–279, 2011.

[9] M. P. Mattson, "Pathways towards and away from Alzheimer's disease," *Nature*, vol. 430, no. 7000, pp. 631–639, 2004.

[10] Y. Enokido, "New aspects of neuronal cell death: the molecular basis of neurodevelopmental and neurodegenerative disorders," *Nihon Shinkei Seishin Yakurigaku Zasshi*, vol. 32, no. 1, pp. 31–35, 2012.

[11] J. B. Martin, "Molecular basis of the neurodegenerative disorders," *The New England Journal of Medicine*, vol. 340, no. 25, pp. 1970–1980, 1999.

[12] G. Pepeu, M. G. Giovannini, and L. Bracco, "Effect of cholinesterase inhibitors on attention," *Chemico-Biological Interactions*, vol. 203, no. 1, pp. 361–364, 2013.

[13] D. Aarsland, U. P. Mosimann, and I. G. McKeith, "Role of cholinesterase inhibitors in Parkinson's disease and dementia with Lewy bodies," *Journal of Geriatric Psychiatry and Neurology*, vol. 17, no. 3, pp. 164–171, 2004.

[14] R. T. Bartus, R. L. Dean III, B. Beer, and A. S. Lippa, "The cholinergic hypothesis of geriatric memory dysfunction," *Science*, vol. 217, no. 4558, pp. 408–417, 1982.

[15] L. M. Teles-Grilo Ruivo and J. R. Mellor, "Cholinergic modulation of hippocampal network function," *Frontiers in Synaptic Neuroscience*, vol. 5, article no. 2, 2013.

[16] D. K. Lahiri and M. R. Farlow, "Review: Cholinesterase inhibitors have a modest effect on neuropsychiatric and functional outcomes in Alzheimer's disease: Commentary," *Evidence-Based Mental Health*, vol. 6, no. 3, p. 94, 2003.

[17] H. Ha, J. K. Lee, and H. Y. Lee, "Safety evaluation of *Yukmijihwang-tang*: assessment of acute and subchronic toxicity in rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 672136, 8 pages, 2011.

[18] S. Lee, J. Park, H. Lee, and K. Kim, "Development and validation of Yin-Deficiency questionnaire," *American Journal of Chinese Medicine*, vol. 35, no. 1, pp. 11–20, 2007.

[19] S.-H. Lee, "A Study of 123 prescriptions in high-chepter from Bang-Yak-Hap-Pyun (Mainly Basic-prescriptions," *Herbal Formula Science*, vol. 17, no. 1, pp. 1–16, 2009.

[20] S. Rho, M. Kang, B. Choi et al., "Effects of Yukmijihwang-tang derivatives (YMJd), a memory enhancing herbal extract, on the gene-expression profile in the rat hippocampus," *Biological & Pharmaceutical Bulletin*, vol. 28, no. 1, pp. 87–93, 2005.

[21] H.-I. Choi, H.-W. Lee, T.-M. Eom et al., "A traditional Korean multiple herbal formulae (Yuk-Mi-Jihwang-Tang) attenuates acute restraint stress-induced brain tissue oxidation," *Drug and Chemical Toxicology*, vol. 40, no. 2, pp. 125–133, 2017.

[22] J.-S. Lee, H.-G. Kim, H.-W. Lee et al., "Hippocampal memory enhancing activity of pine needle extract against scopolamine-induced amnesia in a mouse model," *Scientific Reports*, vol. 5, article no. 9651, 2015.

[23] F. E. Harrison, S. S. Yu, K. L. Van Den Bossche, L. Li, J. M. May, and M. P. McDonald, "Elevated oxidative stress and sensorimotor deficits but normal cognition in mice that cannot synthesize ascorbic acid," *Journal of Neurochemistry*, vol. 106, no. 3, pp. 1198–1208, 2008.

[24] L. B. Gao, X. F. Yu, Q. Chen, and D. Zhou, "Alzheimer's Disease therapeutics: current and future therapies," *Minerva Med*, vol. 107, no. 2, pp. 108–113, 2016.

[25] J. Shen, "Impaired neurotransmitter release in Alzheimer's and Parkinson's diseases," *Neurodegenerative Diseases*, vol. 7, no. 1-3, pp. 80–83, 2010.

[26] P. R. Heckman, C. Wouters, and J. Prickaerts, "Phosphodiesterase inhibitors as a target for cognition enhancement in aging and Alzheimer's disease: a translational overview," *Current Pharmaceutical Design*, vol. 21, no. 3, pp. 317–331, 2014.

[27] H. Hu, R. Zhang, Y. Zhang, Z. Xia, and Y. Hu, "Role of CREB in the regulatory action of sarsasapogenin on muscarinic M1 receptor density during cell aging," *FEBS Letters*, vol. 584, no. 8, pp. 1549–1552, 2010.

[28] S. G. Anagnostaras, G. G. Murphy, S. E. Hamilton et al., "Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice," *Nature Neuroscience*, vol. 6, no. 1, pp. 51–58, 2003.

[29] S.-Z. Wang, S.-Z. Zhu, D. C. Mash, and E. E. El-Fakahany, "Comparison of the concentration of messenger RNA encoding four muscarinic receptor subtypes in control and Alzheimer brains," *Brain Research*, vol. 16, no. 1-2, pp. 64–70, 1992.

[30] T. Mantamadiotis, T. Lemberger, and S. C. Bleckmann, "Disruption of CREB function in brain leads to neurodegeneration," *Nature Genetics*, vol. 31, no. 1, pp. 47–54, 2002.

[31] B. Lee, R. Cao, Y.-S. Choi et al., "The CREB/CRE transcriptional pathway: protection against oxidative stress-mediated neuronal cell death," *Journal of Neurochemistry*, vol. 108, no. 5, pp. 1251–1265, 2009.

[32] S. Kotani, T. Yamauchi, T. Teramoto, and H. Ogura, "Pharmacological evidence of cholinergic involvement in adult hippocampal neurogenesis in rats," *Neuroscience*, vol. 142, no. 2, pp. 505–514, 2006.

[33] B. Radwanska-Wala, E. Buszman, and D. Druzba, "Reactive oxygen species in pathogenesis of central nervous system diseases," *Wiad Lek*, vol. 61, no. 1-3, pp. 67–73, 2008.

[34] B. D. Watson, "Evaluation of the concomitance of lipid peroxidation in experimental models of cerebral ischemia and stroke," *Progress in Brain Research*, vol. 96, pp. 69–95, 1993.

[35] M. Balkaya, V. Prinz, F. Custodis et al., "Stress worsens endothelial function and ischemic stroke via glucocorticoids," *Stroke*, vol. 42, no. 11, pp. 3258–3264, 2011.

[36] S. Choi, *Korean Medical Pathology, Seoul: Iljoongsa*, Iljoongsa, Seoul, 1997.

[37] B.-S. Koo, W.-C. Lee, Y.-C. Chang, and C.-H. Kim, "Protective effects of alpinae oxyphyllae fructus (*Alpinia oxyphylla* MIQ) water-extracts on neurons from ischemic damage and neuronal cell toxicity," *Phytotherapy Research*, vol. 18, no. 2, pp. 142–148, 2004.

[38] S.-H. Shi, X. Zhao, B. Liu et al., "The effects of sesquiterpenes-rich extract of Alpinia oxyphylla Miq. On amyloid- β -induced cognitive impairment and neuronal abnormalities in the cortex and hippocampus of mice," *Oxidative Medicine and Cellular Longevity*, vol. 2014, Article ID 451802, 2014.

[39] X. Yu, L. An, Y. Wang, H. Zhao, and C. Gao, "Neuroprotective effect of Alpinia oxyphylla Miq. fruits against glutamate-induced apoptosis in cortical neurons," *Toxicology Letters*, vol. 144, no. 2, pp. 205–212, 2003.

[40] D. A. Drachman and J. Leavitt, "Human memory and the cholinergic system: a relationship to aging?" *JAMA Neurology*, vol. 30, no. 2, pp. 113–121, 1974.

[41] J. J. Palop and L. Mucke, "Amyloid-B-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks," *Nature Neuroscience*, vol. 13, no. 7, pp. 812–818, 2010.

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Research Article

Integrative Medicine for Postoperative Patients: A Survey of Korean Medicine Doctors

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The purpose of this survey was to document the experience of Korean Medicine doctors (KMD) who provided postoperative care to patients through integrative medicine and to understand their opinions about integrative medicine utilization. Three researchers (two with a KMD license) of the Korea Institute of Oriental Medicine conducted the survey. The questionnaire was distributed via e-mail to the 17,041 members of the Association of Korean Medicine in 2015. In total, 487 KMD answered the questionnaires. The majority of respondents worked in a Korean Medicine (KM) clinic, KM hospital, or long-term care hospitals (94.7%). The respondents mostly treated patients after musculoskeletal (26.7%), spinal (23.7%), or neuropathic surgery (22.2%). Patients predominantly experienced pain (23.0%), fatigue and tiredness (17.4%), delayed scar recovery (13.7%), and paralysis (13.0%). We analyzed subgroups in accordance with institution of employment, specialization, and clinical experience. Most KMD wanted to utilize integrative medicine for postoperative care of patients (92.6%). Moreover, a relatively active collaboration was noted in long-term care hospitals (mean rate: 60.73% [95% CI: 42.25 to 79.20]). Further studies and clinical trials are needed to determine whether integrative medicine is essential for providing postoperative care to Korean patients.

1. Introduction

Integrative medicine is a collaborative approach to patient care that involves "bringing conventional and complementary approaches together in a coordinated way" and suggests a patient-centered treatment [1]. Complementary approaches mainly indicate complementary and alternative medicine, which encompass "a group of diverse medical and healthcare interventions that are not generally included under conventional medicine [2]."

In Korea, Korean Medicine (KM) plays a pivotal role in complementary therapies. KM involves the same clinical techniques as East Asian traditional medicine, such as traditional Chinese medicine (TCM). However, it also has distinct characteristics, including Sasang typology, Saam acupuncture, Chuna therapy, and pharmacopuncture. In recent times, KM has adapted techniques of western medicine for evaluating the efficacy of KM by known biomarkers, in order to secure the safety of KM and to validate the efficacy of KM scientifically [3].

Integrative medicine has been adapted to treat chronic diseases and postoperative adverse effects in Korea. A Stroke and Neurological Disorders Center in a KM hospital has conducted integrative stroke care, in which KM and physical medicine, involving rehabilitation, are used after neurosurgery. The number of inpatients with stroke treated using such integrative medicine has incrementally increased [4]. In addition, the KM hospitals particularly provide care for patients with musculoskeletal disorders in Korea and several US hospitals use integrative medicine for the treatment of patients with spinal disorders [5]. Clinical trials have also been conducted using different methodologies including the protocol for a prospective observational study using integrative medicine in children with cerebral palsy, protocol

of three-armed randomized controlled trial for symptomatic lumbar spinal stenosis, and case series of prescribing herbal medicine and gefitinib for non-small-cell lung cancer patients [6–8].

Several surveys have been conducted on the use of treatments employing integrative medicine [5, 9, 10]; however, there has been no survey on the use of integrative medicine for postoperative care among patients in Korea. The present survey aimed to discern the methods for treating postoperative symptoms on the basis of KM and to examine the experiences and insights of KMD on the use of integrative medicine to treat patients with these symptoms.

2. Materials and Methods

2.1. Subjects. All KMD who signed up to the website of the Association of Korean Medicine (AKOM) were the subjects of this research. The survey was distributed to these KMD via e-mail on October 13, 2015, and again on October 20, 2015. A mobile phone text message, encouraging KMD to complete the survey, was sent to AKOM members simultaneously with the second survey e-mail. The survey acceptance time was 15 days.

2.2. Contents of Survey. Three researchers of the Korea Institution of Oriental Medicine (KIOM) developed the survey protocol and questions. Two of the members were certified KMD.

The questionnaire comprised 31 items, including specific questions, and was based on a Likert five-point scale. The survey topic of integrative medicine was divided into 3 parts. The first part asked the respondent's basic information (sex, age, type, and location of workplace, years of clinical practice experience, certification as a KMD, educational background, highest qualification, number of patients per week, and dual-certification as conventional medicine doctor and KMD).

The second part included general questions about integrative medicine. In this survey, integrative medicine in Korea was defined based on 3 concepts proposed by the Ministry of Health and Welfare in Korea (MOHW) and interorganizational collaboration in Canada [11, 12]. The first concept, latent collaboration, represents a less formal form of association between western medicine and KM and involves the use of routine common medications. The second concept entails developing collaboration between western medicine and KM, where the respective specialties are retained while working synergistically to achieve treatment efficacy. The third is active collaboration and involves optimizing the strengths of Korean and western medicine while minimizing the weaknesses.

Consequently, each of these methods could be compounded to become a new treatment approach. To summarize, this part of the survey determined KMD's current integrative medicine intervention status and monitored their requirements with respect to integrative medicine. There were seven questions in this part of the survey (necessity of integrative medicine, current status of integrative medicine usage, the rate of integrative medicine implementation, diseases frequently treated using integrative medicine, patients' visiting style, and the ideal style of integrative medicine).

Finally, the third part of the survey involved questions about the postoperative use of integrative medicine. The surgeries for which postoperative care was most often provided by KMD were ranked, and subquestions were included about integrative medicine (treatment frequency and types of acupuncture, formulations of herbal medicine and its coverage of health insurance, and additional therapies, such as moxibustion and cupping) and were added.

The full survey text can be accessed in Appendix 1 in Supplementary Material available online at https://doi.org/10.1155/2017/4650343.

2.3. Statistical Methods. A statistician analyzed the frequencies and used the t-test to compare data, by implementing SPSS version 20 (IBM Corp., Armonk, NY). A P value of <0.05 was considered for statistical significance.

2.4. Ethical Approval. This survey was approved by the institutional review board of KIOM in September 2015 (I-1509/005-002).

3. Results

3.1. Clinical Demographics. The number of certified KMD in 2014 was 22,074 [13]. A total of 17,041 members, subscribed in the website of AKOM, were approached to complete the survey, and 487 responded; that is, the response rate was 2.86%. The respondents' sex ratio was convergent with that in the KMD population and the composition of each working institute where respondents worked was equivalent to that of the survey demographics [13]. Respondents' mean age was 41.80 years, and the proportion of male KMD was 4 times greater than that of female KMD. A KM clinic was the most common place of employment (Table 1).

3.2. General Integrative Medicine Utilization and Needs. Approximately 90% of respondents answered that integrative medicine should be used to expand the usage of KM. However, only 34% of KMD had experience in utilizing integrative medicine. A total of 79.3% of KMD working in a KM hospital were treating patients using integrative medicine. In contrast, only 19% of KMD working in a KM clinic utilized integrative medicine. The latent collaboration was the most common type of integrative medicine used by KMD (70.8%). Only 8.4% of participants used the active collaboration type of integrative medicine.

In this survey, approximately 33% of respondents' patients visited a KM clinic or hospital by themselves after they had received treatments in conventional medicine institutions. Moreover, 32% of respondents' patients visited a KM clinic as first recourse. The most prevalent integrative medicine type was latent collaboration, based on patients' request (38%), while active collaboration was marginally attempted (1.8%). In contrast to a real-world setting, more than a half of the respondents (56%) wanted to treat patients using a developing collaboration approach, that is, in cooperation with

Table 1: Demographics of 487 KMD respondents and all KMD in 2014.

	Respondents	Total KMD in 2014
Sex (%)		
Male	397 (82)	
Female	90 (19)	
Total	487 (100)	22,074 (100)
Mean age (SD, 95% CI), years	41.8 (8.7, 41.0–42.6)	
Working institute (%)		
Community health center	8 (1.6)	917 (4.2)
KM clinic	348 (71.5)	14,798 (67)
KM hospital	87 (17.9)	1446 (6.6)
Hospital	3 (0.6)	1774 (8)
General hospital	7 (1.4)	25 (0.1)
Long-term care hospital	26 (5.3)	1217 (5.5)
Institution and research center	4 (0.8)	
Others	4 (0.8)	1897 (8.6)
Total	487 (100)	22,074 (100)
Mean clinical experiences (SD), years	14.4 (7.9)	
Major (%)		
Nonspecialist	331 (68)	19,602 (79.8)
KM internal	50 (10)	919 (3.7)
Gynecology	17 (3.5)	208 (0.9)
Pediatrics	5 (1)	91 (0.4)
Ophthalmology/ENT/dermatology	12 (2.5)	140 (0.6)
Sasang	4 (0.8)	134 (0.5)
Rehabilitation	25 (5)	326 (1.3)
Psychology	5 (1)	148 (0.6)
Acupuncture	37 (7.6)	506 (2)
Others	1 (0.2)	2472 (10)
Total	487 (100)	
Final education (%)		
Bachelor's degree	189 (38.8)	
Master's degree	101 (20.7)	
Ph.D.	196 (40.3)	
Others	1 (0.2)	
Total	487 (100)	
Dual certification (%)		
Yes	4 (0.8)	247 (1.0)
No	483 (99.2)	21,827 (99.0)
Total	487 (100)	22,074 (100)
Mean number of patients seen per week (%)		
Less than 50	69 (14.2)	
50–99	90 (18.5)	
100–199	186 (38.2)	
200–299	95 (19.5)	
300–399	31 (6.3)	
400–499	7 (1.4)	
More than 500	6 (1.2)	
N/A	3 (0.6)	
Total	487 (100)	

Table 2: Status of integrative medicine usage by KMD (multiple responses).

Diseases applying integrative medicine (%)	Number of KMD who had experience in using integrative medicine (%)
Musculoskeletal disorders	108 (17.1)
Cardiovascular disorders	63 (10.0)
Neurological disorders	53 (8.3)
Digestive disorders	46 (7.3)
Respiratory disorders	45 (7.2)
Gynecologic disorders	43 (6.8)
Rheumatic diseases	33 (5.2)
Endocrine disorders	32 (5.0)
Mental disorders	30 (4.7)
Allergic disorders	25 (3.9)
Dermatological disorders	25 (3.9)
Urinary system disorders	24 (3.8)
Hematologic and oncologic disorders	23 (3.6)
Otorhinolaryngology disorders	21 (3.3)
Pediatric disorders	15 (2.3)
Infectious diseases	12 (1.9)
Ophthalmological disorders	11 (1.7)
Kidney diseases	10 (1.6)
Oral system disorders	5 (0.7)
Others	5 (0.7)
Total	629 (100)

doctors, after requesting such cooperation. In total, 24.6% of KMD respondents asserted that a conventional doctor and KMD should work together from the beginning. Integrative medicine is most commonly used in musculoskeletal and cardiovascular disorder management (27.2%). (Table 2).

3.3. Integrative Medicine for Postoperative Care. More than 80% of participants who were experienced in using integrative medicine had utilized integrative medicine for postoperative care (81.33%). A total of 135 respondents gave multiple responses to this question, by frequency. The highest frequency of integrative medicine utilization to care postoperative symptoms was for fracture and joint surgery (26.7%), while spinal surgery was ranked first when 135 participants graded their experience frequency for postoperative treatment (ranked first: spinal surgery = 31.1%, fracture and joint surgery = 29.6%, and brain and neuropathy surgery = 28.9%). Spinal surgery was the second most common intervention for which KMD adopted an integrative medicine approach to surgery patients. The third most frequent use of integrative medicine to relieve adverse effects of surgery intervention was for neuropathy and brain disease surgery (22.2%). The most common postoperative adverse effects treated by integrative medicine were pain, paralysis with numbness and dizziness (controlled chiefly by acupuncture), fatigue and tiredness, and delayed scar recovery (treated with herbal medicine; Table 3).

3.4. Classification of Respondents by Place of Employment. The proportion of KMD working in a KM hospital, clinic, or long-term care hospital was 95%. In this survey, the proportion of KMD specifically working in a KM clinic was 71%. Additionally, 80% of members of KM clinics were nonspecialist KMD, while more than 70% of KMD working in KM hospitals and long-term care hospitals were certified as KM specialists.

Respondents from KM hospitals emphasized that integrative medicine is essential for postoperative care (95.4%). Accordingly, KMD in KM hospitals utilized integrative medicine more actively than did those from other institutions (KM hospitals: 79.3%, long-term care hospitals: 69.2%, and KM clinics: 19.0%).

All 3 groups responded that latent collaboration is the most frequent type of integrative medicine implemented. The rate of collaboration by KMD in KM clinics was markedly lower than that in the other 2 groups (KM clinics: 12.4%, KM hospitals: 63.2%, and long-term care hospitals: 61.5%).

The mean frequency of integrative medicine was the highest in long-term care hospitals, that is, 60.73% (95% CI: 42.25 to 79.20). The mean rate of KM hospital was 29.76% (95% CI: 23.46 to 36.05), and that of the KM clinic was the lowest (mean rate: 6.57%, 95% CI: 4.64 to 8.51).

All KMD groups typically treated musculoskeletal, cardiovascular, and digestive diseases. Long-term care hospital KMD cared for patients who had various symptoms. They additionally used integrative medicine in digestive disorders (46.2%, KM hospital: 12.6%, KM clinic: 6.9%), musculoskeletal disorders (61.5%, KM hospital: 47.1%, KM clinic: 13.2%), and neurological disorders (38.5%, KM hospital: 24.1%, KM clinic: 13.2%) (Table 4).

The pattern of patients' hospital visits was determined based on multiple responses. In case of long-term care hospitals, patients mostly visited the hospital by themselves after visiting conventional hospitals (94%), but, in KM clinics and hospitals, patients visited the institution as first recourse (98.6% and 100%, resp.). All 3 institutional KMD answered that the ideal type of integrative medicine should be treated after joint discussion. Respondents in KM hospitals and long-term care hospitals usually asked for treatment from doctors in the same institution (KM hospital: 72.5%, $n = 50$, long-term care hospital: 94.4%, $n = 17$), while KMD in clinics were generally requested from another conventional clinic ($n = 55$, 79.7%).

Current integrative medicine execution was different. In KM clinics, KMD usually treated patients on the basis of the patients' own opinions (14.4%). In contrast, KM hospital workers treated patients by consultation with conventional medicine doctors, with no prior discussion (35.6%). In long-term care hospitals, KMD worked together with conventional doctors to perform integrative medicine (34.6%).

KM hospital members commonly had more experience in using integrative medicine for postoperative patients than did those working at long-term care hospitals and KM clinics

Table 3: Frequency of highly used interventions treating postoperative symptoms.

	Acupuncture			Herbal medicine		
	Fracture and joint surgery (%)	Spine surgery	Brain and neuropathy surgery	Fracture and joint surgery	Spine surgery	Brain and neuropathy surgery
Pain	59 (53.2)	52 (51.0)	23 (28.8)	8 (6.8)	7 (8.0)	3 (2.9)
Paralysis and numbness	19 (17.1)	28 (27.5)	34 (42.5)	4 (3.4)	4 (4.5)	8 (7.6)
Delay of scar recovery	14 (12.6)	10 (9.8)	5 (6.3)	34 (29.1)	23 (26.1)	4 (3.8)
Fatigue and tiredness	3 (2.7)	3 (2.9)	N/A	30 (25.6)	34 (38.6)	35 (33.3)
Dizziness	N/A	N/A	5 (6.3)	N/A	1 (1.1)	23 (21.9)
Total	111 (100)	102 (100)	80 (100)	117 (100)	88 (100)	105 (100)

Table 4: Frequently treated diseases using integrative medicine in KM clinics and hospitals.

	KM clinic	KM hospital	Long-term care hospital
Musculoskeletal disorders	46 (15.0)	41 (18.8)	16 (19.3)
Cardiovascular disorders	27 (8.8)	26 (11.9)	9 (10.8)
Digestive disorders	24 (7.8)	11 (5.0)	12 (14.5)
Gynecologic disorders	21 (6.9)	15 (6.9)	3 (3.6)
Respiratory disorders	20 (6.5)	17 (7.8)	5 (6.0)
Rheumatic diseases	19 (6.2)	7 (3.2)	6 (7.2)
Mental disorders	19 (6.2)	7 (3.2)	4 (4.8)
Dermatological disorders	19 (6.2)	7 (3.2)	N/A
Neurological disorders	17 (5.6)	21 (9.6)	10 (12.0)
Allergic disorders	16 (5.2)	7 (3.2)	2 (2.4)
Endocrine disorders	15 (4.9)	13 (6.0)	3 (3.6)
Otorhinolaryngologic disorders	12 (3.9)	7 (3.2)	1 (1.2)
Ophthalmological disorders	9 (2.9)	3 (1.4)	N/A
Urinary System disorders	9 (2.9)	7 (3.2)	7 (8.4)
Hematologic and oncologic disorders	7 (2.3)	8 (3.7)	3 (3.6)
Pediatric disorders	7 (2.3)	7 (3.2)	1 (1.2)
Kidney diseases	5 (1.6)	4 (1.8)	1 (1.2)
Infectious diseases	5 (1.6)	8 (3.7)	N/A
Oral system disorders	5 (1.6)	1 (0.5)	N/A
Others	4 (1.3)	1 (0.5)	N/A
Total	306 (100)	218	83 (100)

(KM hospitals: 65.5%, long-term care hospitals: 50%, and KM clinics: 15.5%). Commonly performed surgeries were those for bones and fracture, spine, and brain and neuropathies. Patients who underwent brain surgery had been commonly treated in long-term care hospitals (92.9%). Highly treated postoperative patients had similar illnesses, regardless of the institutions involved. KMD in all 3 institutions treated for pain, numbness and paralysis, dizziness, and delay in scar recovery.

The interventions used to treat disease most frequently were acupuncture and herbal medicine (acupuncture—KM clinic: 6.3%, KM hospital: 24.1%, long-term care hospital: 42.3%, Herbal medicine—KM clinic: 4.0%, KM hospital: 19.5%, and long-term care hospital: 11.5%). KMD from all institutions usually used nonbenefit decoctions (the health insurance does not cover the costs of drugs).

3.5. Comparison of KMD Specialists and Nonspecialists. In this survey, more than 30% of the respondents were KMD specialists. Answers of specialists were somewhat different from those of nonspecialists. The specialists' mean age was 39.47 years, which was 3.5 years younger than that of nonspecialists (P value < 0.05). There were 157 KMD specialists in this survey.

KMD specialists tended to answer that integrative medicine is essential to KMD more than did nonspecialists. The proportion of KMD specialists utilizing integrative medicine (51.6%) was twice that of nonspecialists (25.8%).

The KMD specialist group significantly more frequently implemented integrative medicine (19.1%) than did the nonspecialists (12.3%; P value = 0.011).

Specialists provided care for patients with musculoskeletal diseases more often than nonspecialists did (specialists: 32.5%, nonspecialists: 17.9%), while nonspecialists treated digestive diseases more often than did specialists (nonspecialists: 11.8%, specialists: 5.7%).

There were various responses on the ideal integrative medicine. Although both groups answered that joint discussion after inquiries (developing collaboration) was the ideal style of consultation (specialists: 63.7%, nonspecialists: 52.4%), the nonspecialist group preferred joint discussion early on (active collaboration, nonspecialists: 26.1%, specialists: 21.7%).

KMD specialists had more experience in treating postoperative patients: 43.3% of KMD specialist respondents treated patients postoperatively, while only 20.3% of nonspecialists had experience in treating patients postoperatively.

3.6. Grouping Respondents by Clinical Experience. Based on KMD' raw response data, KMD could be divided into 3 groups based on clinical experiences (Group 1: Clinical experience of 0–10 years, Group 2: more than 10 years to 20 years, and Group 3: more than 20 years).

All 3 groups performed integrative medicine using latent, developing, and active collaboration, in sequence. Most of the respondents worked together, but without discussion among each other.

Group 1 KMD additionally treated musculoskeletal diseases (Group 1: 27.1%, Group 2: 18.5%, and Group 3: 19.0%). KMD who had less clinical experience mainly treated brain and neurological disorders (73.2%, 63.8%, and 55.6%), and spinal surgery symptoms (73.2%, 72.3%, and 59.3%). In contrast, gynecological disorders were treated by a clinically, highly experienced group (23.2%, 27.7%, and 48.1%).

4. Discussion

The reputation of KM had improved as a traditional medicine before the domination of Japan, which fostered the use of western medicine in Korea, and KMD had to contend with government to revive KM. Consequently, Korea adopted a dual-medical policy since 1951, after passing of the National Medicine Services Law [14]. However, doctors practicing each of these forms of medicine have conflicting views, due to different conceptions of treatment and environments. As medical doctors and KMD cared for patients independently, patients were confused by the dual care by KMD and western medicine doctors, and patients with chronic diseases were burdened with additional treatment costs [15]. The Medical Service Act was revised in 2009, and the cooperation between hospitals has become constitutional. Correspondingly, integrative medicine has been implemented in the medical field [16].

Prior surveys in KM hospitals, which explained integrative medicine and collaboration between KMD and conventional western medicine doctors, concluded that integrative medicine had two different purposes. The first aim was therapeutic intervention to relieve patients' discomfort. In a university-owned KM hospital, spine, rehabilitation, and rheumatic disorder centers conducted comparatively active collaboration with conventional western medicine doctors. Conventional western medicine doctors mainly consulted with KMD at the behest of the patients and to relieve their pain. The second purpose was for diagnosis, in which KMD consulted with western medicine doctors, as KMD are prohibited from using medical devices, such as X-rays and ultrasound imaging systems, by the Medical Service Act Article 27 in Korea. In the 3 above-mentioned centers, more than half of consultations with conventional medicine doctors were simple diagnostic requests. KMD consulted with conventional western medicine doctors more frequently than the reverse (59% and 41%, resp.) [17]. Another KM hospital investigated KMD who treated car accident patients and collaborated with conventional medicine doctors. The survey concluded that KMD mainly consulted about treatment with conventional western medicine doctors, particularly those in the field of orthopedics and laboratory medicine, in order to obtain an accurate diagnosis with medical devices (manuscript in preparation).

This survey investigated the use of integrative medicine in the postoperative care of patients in Korea. Our research questionnaire enquired about the experience of clinicians with integrative medicine, with a particular focus on the specific intervention period for general disorders, postoperative care, and adverse effects.

More than 40% of the survey respondents had completed their Ph.D. and the proportion of KM specialists had doubled in 2015 [18]. Despite the absence of data on the educational background of KMD in 2015, their responses implied that younger KMD who had longer educational backgrounds in proportion to nonspecialized KMD were more likely to respond to the survey.

Most respondents in the survey considered that integrative medicine is required to treat postoperative patients. However, the practical usage of integrative medicine to control the adverse effects of surgery in KM institutions was insufficient for KMD. Only one-third of KMD had experience to utilize integrative medicine. Specifically, integrative medicine was applied to KM clinics only briefly.

Patients mainly visited KM institutions by themselves at first, or after visiting conventional hospitals without discussion to conventional medical doctors. Collaborations with doctors were attempted after simple inquiries, rather than by joint discussion. Most KMD in the survey wanted to utilize integrative medicine to achieve effective postoperative symptom care. In addition, KMD required active collaboration. However, the application of integrative medicine in the KM field was limited in our survey analysis.

KMD who had experience in caring for patients with integrative medicine also provided postoperative care for their patients by using integrative medicine. They chiefly treated patients with musculoskeletal, cardiac, or neuropathic conditions. KMD largely controlled musculoskeletal, spine, brain, and neuropathy surgeries. Most postoperative patients experienced pain, numbness, fatigue, and delayed scar recovery. Respondents commonly utilized acupuncture,

pharmacopuncture, and herbal medicine decoctions not covered by medicine insurance system to care for postoperative patients. The survey reflected frequently used KM interventions in Korea. In a survey of the use of KM by KMD in patients in 2014, by the Korea Health Industry Development Institute, the pivotal treatment methods were acupuncture (59.2%), herbal medicine decoction (27.6%), and herbal preparation (4.9%) [19].

According to the health insurance statistics from 2014, low back pain (M54.5) was the most frequent among 10 conditions in inpatients in the field of KM; 55,615 patients were diagnosed with this condition. The second most common condition was gonarthrosis (M17), and the third was dislocation, sprain, and strain of joints and ligaments of the lumbar spine and pelvis (S33). The first among 10 diseases in outpatients was also low back pain, which was diagnosed in 4,260,471 patients. The second most common disease was soft tissue disorder, unspecified (M79.9). The third condition was M17 and S33. Patients who visited KM hospitals also suffered from the above diseases. The codes for the disease diagnosis followed ICD-10 2010 version [20].

Most KMD worked in KM clinics, KM hospitals, and long-term care hospitals. In this survey, integrative medicine was predominantly practiced in KM hospitals and long-term care hospitals, which have departments both of KM and of conventional medicine. In KM clinics, cooperation between conventional doctors and KMD was less active than in the other institutions. Since the Medical Service Act related to hiring medical professionals in different institutions had been revised in 2009, cooperation in KM clinics is mainly conducted by requirement of patients, while collaboration in KM hospitals and long-term care hospitals is mainly led by medical professionals [21]. Political intervention is required to ensure active collaboration between KMD and conventional medicine doctors in KM clinics.

Cooperation in long-term care hospitals was based on an understanding of the medical history of collaboration between conventional western medicine and KM in South Korea. Long-term care hospitals are medical institutions established by medical doctors or KMD in order to treat patients over the long term, where more than 30 patients can be hospitalized concurrently, which have largely been established since 2005 [18]. The number of western medicine doctors increased from 1.3 to 2.1 per 100 beds and KMD increased from 0.1 to 0.7, during the period 2005 to 2013 [22]. The number of KMD working in long-term care hospitals was 1244 in 2014. More than 90% of KMD who worked at hospitals were working in the long-term care hospitals. In 2008, the medical insurance fee in long-term care hospitals changed to a fixed medical insurance fee per day [23].

Furthermore, collaboration between KMD and conventional medicine doctors can increase the income of long-term care hospitals by means of fee-for-service, while per diem payment is applied for hospitalization in conventional medicine departments situated in long-term care hospitals. Since long-term care hospitals mainly care for patients with chronic diseases and chronically ill patients in Korea have favored KM, collaboration can frequently be implemented [24].

In the case of KM hospitals, the institution can hire medical practitioners to make a diagnosis using methods such as computed tomography, magnetic resonance imaging, radiography, and ultrasonography, which KMD cannot use given the Medical Service Act, Article 27, in Korea, and technical needs in KM hospitals also support frequent implementation of collaboration. Consequently, integrative medicine can be implemented suitably in KM hospitals and in long-term care hospitals.

The survey assessed KMD specialist certification. The first KMD specialists were certified in 2002. According to the 2014 KM Chronology, around 11% of KMD were specialists [24]. In this survey, 20% of respondents were KMD specialists. They implemented integrative medicine in postoperative patients more often than nonspecialists, emphasizing the necessity for integrative medicine.

Respondents with more clinical experience tended to treat patients with gynecological disease more often than did the respondents with less clinical experience. KMD working in KM clinics also treated patients with gynecological disease. As the relationship between clinical experience and the frequency of treating gynecological disorders cannot be unambiguously defined by the present survey, further studies are needed.

There are several limitations associated with this survey. First, this was a cross-sectional study and used subjective indicators, such as the rate of implementation of integrative medicine in the comprehensive treatment provided by KMD. Second, the answer rate was relatively low in proportion to the number of members in AKOM, as compared to other survey results [9]. E-mail answers partly represented the respondents' real-time interventions. Consequently, the survey was not analyzed to determine a cause-and-effect relationship between other statistics, and studies such as those using the national health and insurance data.

A previous study concluded that the collaboration rate of long-term care hospitals was low in proportion to the rate of collaboration in KM hospitals, while both institutions actively introduced collaboration after the hiring of both KMD and conventional medicine doctors was authorized in both types of institutions [21]. This survey conclusion was not identical to that of a prior study, but we could not analyze the correlation of these two studies and our survey questions partly reflect the real world of integrative medicine in long-term care hospitals. Additional studies need to determine the real status and depth of collaboration in long term care hospitals and compare the strengths and shortfalls of this and previous studies in this field.

Further qualitative studies based on large datasets are required to determine the real impact of KM. Additionally, the recognition by non-KMD of the utility of integrative medicine can be improved. We believe that clinical trials and observational studies can be conducted to reveal the efficiency of integrative medicine in the postoperative care of patients.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

[1] J. Weeks, "The New USA NIH Strategic Plan for Complementary and Integrative Health: Interview with Josephine Briggs, MD: Director, National Center for Complementary and Integrative Health (Part 1)," *Alternative and Complementary Therapies*, vol. 22, no. 6, pp. 251–254, 2016.

[2] NIH, "National Center for Complementary and Alternative Medicine. Strategic plan 2011-2015," https://nccih.nih.gov/about/plans/2011/introduction.htm, 2011.

[3] W. Park, S. Mollahaliloglu, V. Linnik, and H. Chae, "Scientific evidence for Korean medicine and its integrative medical research," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 967087, 2 pages, 2015.

[4] S. U. Park, S. Y. Cho, J. M. Park et al., "Integrative treatment modalities for stoke victims in Korea," *Complementary Therapies in Clinical Practice*, vol. 20, no. 1, pp. 37–41, 2014.

[5] Y. S. Shin, J. S. Shin, J. Lee et al., "A survey among Korea Medicine doctors (KMDs) in Korea on patterns of integrative Korean Medicine practice for lumbar intervertebral disc displacement: Preliminary research for clinical practice guidelines," *BMC Complementary and Alternative Medicine*, vol. 15, no. 1, article no. 432, 2015.

[6] J. E. Yoo, Y. J. Yun, Y. B. Shin et al., "Protocol for a prospective observational study of conventional treatment and traditional Korean medicine combination treatment for children with cerebral palsy," *BMC Complementary and Alternative Medicine*, vol. 16, no. 1, article no. 172, 2016.

[7] K. Lee, Y. S. Kim, C. G. Son et al., "Combination therapy of gefitinib and Korean herbal medicines could be a beneficial option for patients with non-small cell lung cancer," *Journal of Pharmacopuncture*, vol. 19, no. 3, pp. 259–263, 2016.

[8] K. Kim, K. M. Shin, J. H. Lee et al., "Nonsurgical Korean Integrative Treatments for Symptomatic Lumbar Spinal Stenosis: A Three-Armed Randomized Controlled Pilot Trial Protocol," *Evidence-based Complementary and Alternative Medicine*, vol. 2016, Article ID 2913248, 2016.

[9] K. H. Bae, J. A. Lee, K. H. Park, J. H. Yoo, Y. Lee, and S. Lee, "Cold Hypersensitivity in the Hands and Feet May Be Associated with Functional Dyspepsia: Results of a Multicenter Survey Study," *Evidence-based Complementary and Alternative Medicine*, vol. 2016, Article ID 8948690, 2016.

[10] B. Park, K. J. Yun, J. Jung et al., "Conceptualization and utilization of blood stasis syndrome among doctors of Korean medicine: results of a web-based survey," *American Journal of Translational Research*, vol. 6, no. 6, pp. 857–868, 2014.

[11] D. D'Amour, L. Goulet, R. Pineault, J. F. Labadie, and M. Remondin, "Comparative study of inter-organizational collaboration in four health regions and its effects: the case of perinatal services," *Ontario: Canadian Health Services Research Foundation*, 2003.

[12] MOHW, "2014 Oriental Medicine R&D Industry Information of new certification parts," 2014.

[13] MOHW, "2016 Health and Welfare Statistics Chronology," no. 62, 2016.

[14] K. Y. Jung and C. Y. Lee, "Debate on license System for Korean medicine practitioners while establishing the National Medicine Services law in 1951: Based on stenographic records at 11th Provisional National Assembly," *Korean Journal of Oriental Physiology and Pathology*, vol. 26, no. 5, pp. 588–598, 2012.

[15] D. H. Kim and K. H. Lee, "Utilizing patterns and attitude on collaborating care of Korean Traditional Medicine and Western medicine among cerebral apoplexy patients," *Korea Journal of Hospital Management*, vol. 9, 2002.

[16] T. H. Lee, "Concepts of Integrative Medicine and its Relationship with TKM," *Korea Journal of Oriental Medicine*, vol. 17, no. 3, pp. 9–15, 2011.

[17] S. H. Choi, "[Creation 21] A precedent study on developing evidence-based East-West collaborative clinical models," Kyung Hee University, pp. 271–397, 2011.

[18] MOHW, "2015 Health Insurance Statistics Chronology," 2015.

[19] KHIDI, "Research on Usage of Korean Medicine and Herbal Medicine," 2014.

[20] KHIDI, "A White Book to Health Industry," 2014.

[21] M. J. Park, "Performance of collaboration between Korean medicine and western medicine : utilization and quality of care for stroke patients," 2016.

[22] H. S. Ahn, "Patient care and management in long term care hospitals," Korean Medical Association, 2015.

[23] W. S. Jang, "Portrait of Korean medicine through national statistics," *The Minjok Medicine News*, 2015.

[24] KIOM, "2014 Year Book of Traditional Korean Medicine," 2014.

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Research Article

The Effects of Chunghyul-Dan, an Agent of Korean Medicine, on a Mouse Model of Traumatic Brain Injury

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Chunghyul-Dan (CHD) is the first choice agent for the prevention and treatment of stroke at the Kyung Hee Medical Hospital. To date, CHD has been reported to have beneficial effects on brain disease in animals and humans, along with antioxidative and anti-inflammatory effects. The aim of this study was to evaluate the pharmacological effects of CHD on a traumatic brain injury (TBI) mouse model to explore the possibility of CHD use in patients with TBI. The TBI mouse model was induced using the controlled cortical impact method. CHD was orally administered twice a day for 5 d after TBI induction; mice were assessed for brain damage, brain edema, blood-brain barrier (BBB) damage, motor deficits, and cognitive impairment. Treatment with CHD reduced brain damage seen on histological examination and improved motor and cognitive functions. However, CHD did not reduce brain edema and BBB damage. In conclusion, CHD could be a candidate agent in the treatment of patients with TBI. Further studies are needed to assess the exact mechanisms of the effects during the acute-subacute phase and pharmacological activity during the chronic-convalescent phase of TBI.

1. Introduction

Traumatic brain injury (TBI) is a brain pathology induced by mechanical forces and can be divided into two pathological states, primary and secondary brain damage. The former is characterized by contusion and hemorrhage induced by mechanical forces, such as shearing, tearing, or stretching at the beginning of brain damage. The latter is characterized by delayed neuronal loss and neurological dysfunction triggered by primary brain damage. The pathophysiological mechanisms in secondary brain damage have been well documented [1, 2]. Several studies of various mechanisms in the past decades have shown no significant improvement in TBI treatment [3, 4].

Therefore, it is important to continue developing therapeutic interventions that could be helpful in attenuating the deleterious results of TBI and to search for other interventions used in the traditional medical clinics of East Asia that could be used in the treatment of TBI. In research examining traditional medicines, various studies have been conducted to examine the potential of traditional medicines as clinical interventions for TBI. To date, several herbs and prescriptions of traditional medicines, including Drynariae Rhizoma [5] and MLC901 [6], have been shown to ameliorate the effects of TBI [5–9].

Chunghyul-Dan (CHD) is a encapsulated agent made of an 80% ethanol extract of a preparation composed of Scutellariae Radix, Phellodendri Cortex, Gardeniae Fructus, and Rhei Radix et Rhizoma. It is prescribed most frequently for the prevention, treatment, or inhibition of secondary attacks in patients with stroke in Kyung Hee University Korean Medical Hospital [10, 11].

CHD has been reported to have various pharmacological effects. It has been shown to have beneficial effects on the vascular system, including hypocholesterolemic effects in hyperlipidemic rats and humans [12, 13], inhibitory effects on vascular cell adhesion molecule-1, and stimulatory effects on NO production in human endothelial cells [14]. It has

also shown anti-inflammatory effects, including inhibitory effects on inducible nitric oxide synthase, prostaglandin E2 production in lipopolysaccharide-stimulated RAW264.7 macrophage cells [15], and ameliorating effects on swelling by reducing the mRNA levels of cyclooxygenase-2, interferon-gamma, and interleukin- (IL-) 4 in oxazolone-induced mouse ear dermatitis [16].

Regarding neurological diseases, it was shown to protect PC12 cells and rat primary hippocampal cells against β-amyloid (Aβ) oligomer 1–42 toxicity by inhibiting NO, tumor necrosis factor-α, and IL-1β production in microglial cells [17], protecting the brain damage and cognitive impairment against Aβ oligomer 1–42-induced brain damage by inhibiting the activation of astrocytes and microglia [17]. It also showed a neuroprotective effect against hypoxia-reoxygenation-induced neuroblastoma 2a cell damage via an antiapoptotic mechanism [18]. Clinical studies of CHD have shown beneficial effects in patients with stroke. Briefly, it was shown to decrease systolic blood pressure in stroke patients with stage 1 hypertension [19], improved arterial stiffness in patients with increased pulse wave velocity [20], and reduced the odds ratio of stroke recurrence by 77% compared with antiplatelet agent-managed controls [11].

Taking together the pathological mechanisms of TBI and the pharmacological reports of CHD, we proposed Chunghyul-Dan (CHD), an agent of Korean medicine, as a possible intervention for patients with TBI along with stroke. Thus, we evaluated the pharmacological effects of CHD in a TBI mouse model. A TBI mouse model induced by controlled cortical impact, which is a well-known model, was used to evaluate the effects of CHD on histological injury, blood-brain barrier (BBB) damage, brain edema, and functional deficits.

2. Materials and Methods

2.1. CHD Preparation. CHD was obtained from Kyung Hee Medical Hospital (Code: HH333, Seoul, Korea), which was the same preparation used for the treatment of patients in the hospital. The preparation and standardization of CHD are well described [11, 15, 17–19]. Briefly, each dried component herb including Scutellariae Radix, Coptidis Rhizoma, Phellodendri Cortex, Gardeniae Fructus, and Rhei Radix et Rhizoma was extracted by boiling with 80% ethanol for 2 hours and then mixed in 4 : 4 : 4 : 1 after evaporation and lyophilization, respectively.

2.2. Animals. All surgical procedures were approved by the Kyung Hee University Institutional Animal Care and Use Committee (KHUASP(SE)-15-102). ICR mice (DaeHan BioLink, Korea) were acclimatized for 1 week to controlled temperature conditions (22 ± 2°C), with constant humidity, and a 12 h light/dark cycle. Food and water were available ad libitum. Mice were deprived of food overnight with free access to water before surgery.

2.3. TBI Induction. The TBI mouse model was induced by a modified controlled cortical impact (CCI) protocol [21]. Briefly, mice (25–30 g) were anesthetized with isoflurane and placed on a stereotaxic frame. A 4 mm circular craniotomy was performed on the right hemisphere by using an electric drill (−2 mm anteroposterior and 2 mm mediolateral to the bregma). Trauma was induced with an electric impact device (Leica, USA) using a rounded impact tip (2.5 mm) at a velocity of 2 m/sec with a depth of 2 mm and a duration time of 3 msec. After impact, the surgery site was recovered by the skull and glue applied for fixation. Body temperature was monitored using a rectal thermometer and maintained at 37 ± 0.5°C during surgery by using a heating pad. Postoperatively, mice were allowed to recover in a cage. Sham-operated animals underwent craniotomy without impact.

2.4. Experimental Grouping and CHD Treatment. Mice were divided into six groups (sham, vehicle, 30, 100, 300 mg/kg CHD, and 45 mg/kg minocycline [22]) for the assessment of histological damage and seven groups by adding normal group to the six groups mentioned above for assessment of behavioral studies, brain edema, and BBB damage (n = 8). Normal group and sham-operated group were included for comparison in the behavioral studies. The extract was suspended in distilled water (DW) and daily administered orally at 3.3 mL/kg for the first 5 d after TBI induction. Minocycline was dissolved in normal saline and injected intraperitoneally. Vehicle-treated mice were given the same volume of DW.

2.5. Motor Function Assessment. Motor function was assessed using a beam walking test and balance beam test on 0 d (day), 3 d, and 7 d after induction.

The beam walking test was performed using a previously described method with minor modification [23]. A wooden rectangular bar (1 cm wide, 100 cm long, and 50 cm high) connected the start platform and goal box with a 10 cm opening. Mice underwent training sessions prior to TBI to learn to transverse the beam and enter the goal box. Data were obtained by measuring the mean transversal time and foot fault time in five trials.

The balance beam test was performed using a previously described method with some modification [23]. Briefly, mice were placed on the middle of a wooden rectangular bar (5 mm wide, 100 cm long, and 50 cm high) and scored as follows: mice unable to stay on the beam for 30 sec, 0 points; mice able to stay on the beam for 30 sec, 1 point; mice able to turn to the left or right side of the beam without walking, 2 points; mice able to turn left or right and walk on the beam with more than one step, 3 points; mice able to traverse the beam with more than 50% of foot slip of the affected hind limb, 4 points; mice able to transverse the beam with less than 50% of foot slip of the affected hind limb, 5 points; and mice able to traverse the beam with not more than one foot slip, 6 points.

2.6. Cognitive Function Assessment: Novel Object Recognition (NOR) Test. Cognitive function was measured using an NOR test at 8 d after TBI induction. The test was performed according to a previously described method with minor

modifications [24]. Mice were placed in a black, wooden, no-top square box (45 × 45 cm size, 25 cm high walls) for 30 min daily for 3 d before test. On the trial day, mice were placed into the box with two old objects for 5 min and taken out for 1 hour. They were then placed back in the box with one new object and one old object. Recognition index was calculated as follows:

$$\text{Recognition index} = \frac{(\text{time spent exploring the new object} - \text{time spent exploring the old object})}{(\text{total time spent exploring both objects})}. \quad (1)$$

2.7. Measurement of Brain Injury. The brains were fixed by perfusing with 4% paraformaldehyde at 8 d after induction and cut into 30 μm sections with a Cryocut (Carl Zeiss, Germany) and stained with hematoxylin and eosin (H&E). The damaged area (%) was calculated compared with the intact hemisphere of each mouse using Image J software (NIH, USA).

2.8. Brain Water Contents Assay. Mice were sacrificed 48 hours after TBI induction and their brains were quickly removed and divided into damaged and nondamaged hemispheres. The wet weight of each hemisphere was measured on a chemical balance (Mettler HL52; Ohaus Co., NJ, USA) within 90 s of isolation. After drying the brain in an oven at 105°C for 48 hours, the dry weight was then obtained. The water content of each hemisphere was calculated as [wet weight − dry weight]/wet weight × 100.

2.9. Evans Blue (EB) Leakage Assay. EB leakage was analyzed 48 hours after TBI using a previously reported method [25], with some modifications. EB dye (2%, 5 ml/kg body weight; Sigma-Aldrich) was slowly injected into the tail vein 44 hours after TBI and allowed to circulate for 4 hours. Mice were then anesthetized and perfused with 15 mL 0.1 M phosphate buffer. After brain isolation, the pons and olfactory bulb were removed and the brain was immediately separated into ipsilateral and contralateral hemispheres. The weight of each hemisphere was measured; 2.5 times-concentrated (versus weight/volume) formamide (Sigma-Aldrich) was added and the hemispheres were homogenized. Each homogenized hemisphere was incubated at 60°C for 18 h and then centrifuged at 20,000g for 30 min. The absorption of the supernatant was measured at 610 nm with a spectrophotometer. A standard curve of EB in blank formamide was used to convert absorbency into concentration of EB dye. Data are presented as μg of EB dye per gram tissue [26].

2.10. Statistical Analysis. All results are presented as mean ± SEM and were compared between groups using a one-way ANOVA followed by a post hoc Dunnett test. P values < 0.05 were considered statistically significant.

3. Results

3.1. Protective Effects of CHD in TBI Mouse Model. The H&E-stained brain sections of the vehicle-treated group showed damage mainly in the parietal cortex that was closely related to motor function. The external capsule and corpus callosum were also damaged. Minocycline and CHD-treated groups showed less damage in those areas (Figure 1(a)). The damage in the vehicle-treated group involved 7.3% of hemisphere, while the minocycline-treated group had damage to 3.7% of the hemisphere ($P < 0.05$, Figure 1(b)). The CHD 30 and 100 mg/kg treated groups demonstrated damage in 4.3% and 3.6% of the hemisphere, respectively ($P < 0.05$, Figure 1(b)). However, the group treated with 300 mg/kg did not show any significant difference from the vehicle-treated group (5.7% of hemisphere damaged, Figure 1(b)).

3.2. The Effects of CHD on Brain Water Contents (BWC). The BWC of the vehicle-treated group 48 hours after TBI in the damaged hemisphere was 81.6%, while normal and sham group showed 79.0% and 79.4%, respectively. The minocycline and CHD-treated groups showed a tendency of reduced BWC compared with the vehicle-treated group. Although CHD showed a dose-dependent effect on BWC, it did not have any significant effect (80.4%, 80.6%, and 80.5% BWC in the 30, 100, and 300 mg/kg treated groups, resp.). Minocycline treatment also did not lead to a significant reduction in BWC (Figure 2).

3.3. The Effects of CHD on EB Leakage. The vehicle-treated group had increased EB leakage compared with the sham group (9.63 versus 0.38 μg/g tissue) and normal group showing no EB leakage. Minocycline treatment led to reduced EB leakage compared with that of the vehicle-treated group (5.33 μg/g tissue, $P < 0.01$). CHD showed a dose-dependent effect on EB leakage; however, it did not reach significance (9, 8.16, and 8 μg/g tissue in the 30, 100, and 300 mg/kg treated groups, resp., Figure 3).

3.4. Effects of CHD Treatment on Beam Walking and Balance Beam Tests. In the beam walking test, the vehicle-treated group demonstrated increased latency and number of foot faults 3 d after TBI induction. These recovered to the normal range within 7 d. The sham and normal groups did not show any functional deficits. The minocycline-treated group had a change in the number of foot faults at 3 d ($P < 0.01$) and 7 d ($P < 0.05$) after TBI but not latency. All CHD-treated groups demonstrated decreased latency (3 d, $P < 0.05$) and number of foot faults at 3 d ($P < 0.01$) and 7 d ($P < 0.05$) compared with the vehicle-treated group (Figure 4(a)).

In the balance beam test, the vehicle-treated group had lower scores at 3 and 7 d after TBI compared with the

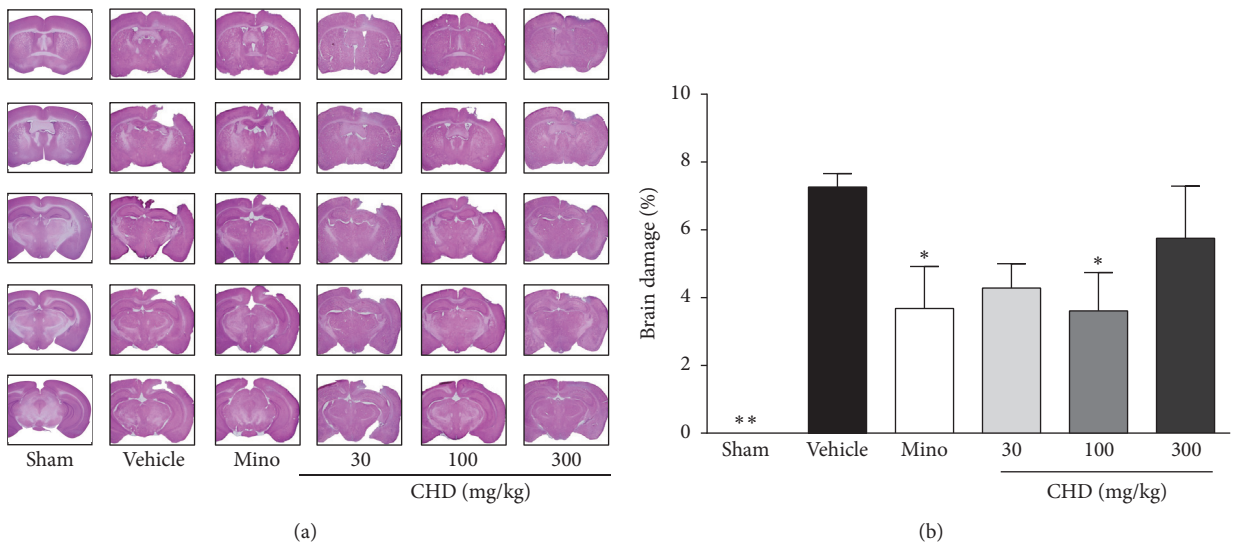

Figure 1: Protective effect of Chunghyul-Dan (CHD) treatment on brain damage in TBI mice model. Brain photos are the representative H&E-stained brain slices (a) and the graph shows brain damage (%) of each group (b). Values represent means ± SEM, $n = 8$. Mino is minocycline 45 mg/kg treated group. ∗ represents statistical difference from vehicle-treated group ($^{*}P < 0.05$, $^{**}P < 0.01$).

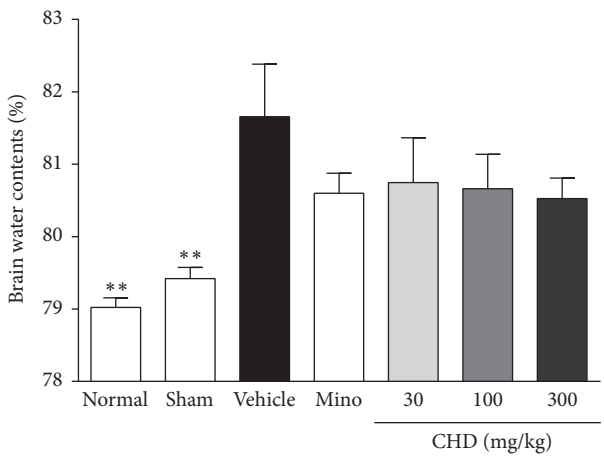

Figure 2: The effect of Chunghyul-Dan (CHD) on brain water content of damaged hemisphere in TBI mouse model. Values represent the mean ± SEM ($N = 8$). Mino is minocycline 45 mg/kg treated group. ∗∗ represents statistical difference from vehicle-treated group ($^{**}P < 0.01$).

sham and normal groups that demonstrated no change in test scores before and after TBI ($P < 0.01$). While the minocycline-treated group had higher scores than the vehicle-treated group at both 3 and 7 d after TBI ($P < 0.01$, Figure 4(b)), the CHD-treated groups also showed significant improvement compared with the vehicle-treated group ($^{*}P < 0.05$, $^{**}P < 0.01$, Figure 4(b)).

3.5. Effects of CHD in the NOR Test. The vehicle-treated group showed a reduced recognition index (%) compared with the sham and normal groups ($P < 0.01$). The CHD-treated groups (30 and 100 mg/kg) had higher recognition indices than the vehicle-treated group ($P < 0.05$). However, the CHD 300 mg/kg treated and minocycline-treated groups did not show any significant differences from the vehicle-treated group (Figure 5).

4. Discussion

In the current study, CHD showed protective effects against brain injury and motor and cognitive functional deficits in a TBI mouse model.

One of the most significant findings of the current study was that CHD ameliorated the effects of brain injury without reducing brain edema or BBB damage. The pathophysiology of TBI is well documented. The brain damage begins with a short-term primary phase caused by direct mechanical force-induced tissue distortion and destruction. It may be worsened by secondary phase brain injury shortly after the initial injury. The secondary phase is characterized by further ischemic or hemorrhagic pathological cascades including inflammation, oxidative stress, and apoptosis [26]. In the current study, the effects of CHD on brain injury might be supported by antioxidative [27, 28], antiapoptotic [18], anti-inflammatory [14, 15, 17, 29], and neuroprotective effects [17, 18, 27], an inhibitory effect of adhesion molecules including vascular cell adhesion molecules [14], and protective effects against stroke [11, 18]. Furthermore, the component herbs also have well-documented activities related to the mechanisms of brain damage [30–37].

However, CHD did not inhibit brain edema or BBB damage. The current results indicated that the protective effect of CHD against brain damage might not be correlated with these phenomena in the acute phase but partly with the subsequent cascades including apoptosis or neuroinflammation.

A second finding of the current study is that CHD improved motor functions as evaluated by the beam walking

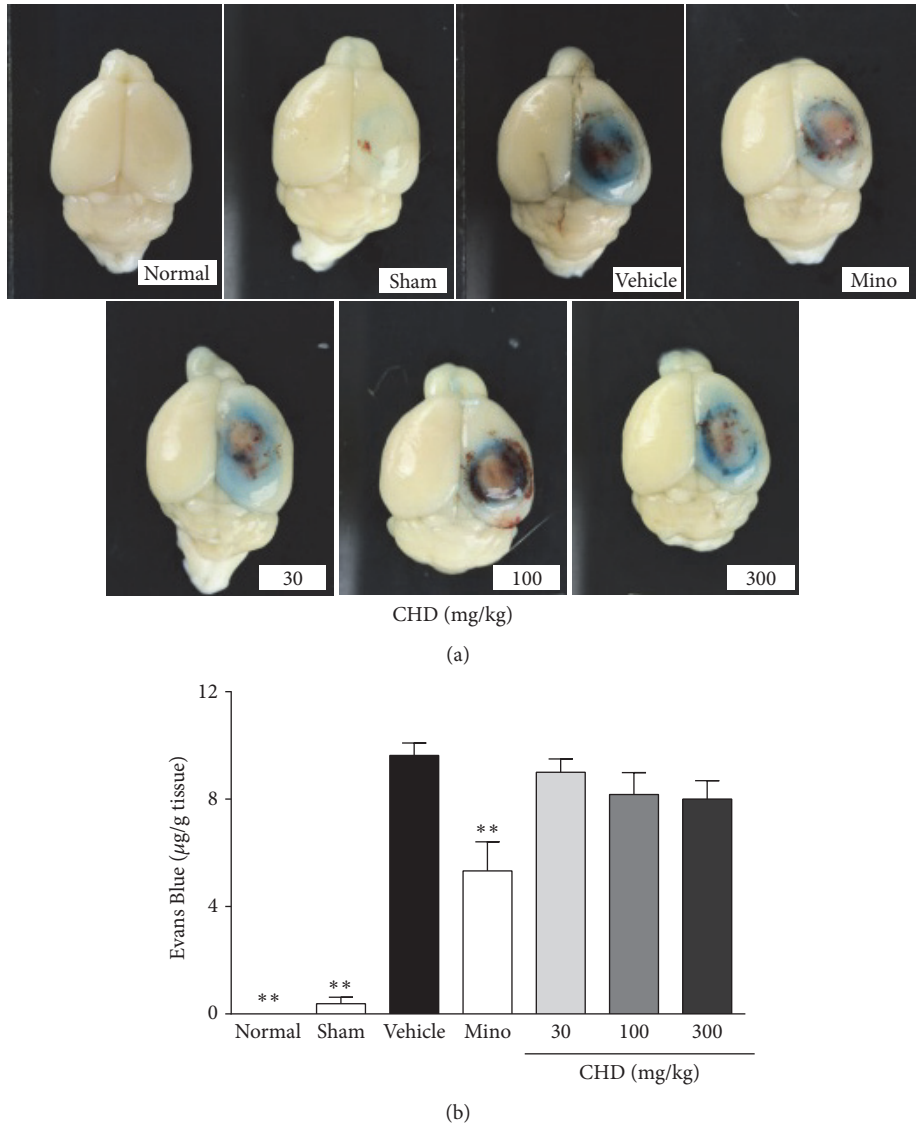

Figure 3: The effect of Chunghyul-Dan (CHD) on Evans Blue leakage (μg/g tissue) in TBI mouse model. Photos are the representative brain of each group after Evans Blue injection (a). The graph shows the Evans Blue contents of each group (μg/g tissue) after quantification (b). Values represent means ± SEM, $n = 8$. Mino is minocycline 45 mg/kg treated group. ∗∗ represents statistical difference from vehicle-treated group ($^{**}P < 0.01$).

and balance beam tests. These tests are well known and have been widely used in studies of various models of brain injury and motor functional deficits to evaluate the sensory motor function, motor coordination, and balance [38–40]. In the current study, the functional tests were performed at 3 and 7 d after TBI induction. Motor function is known to be obviously affected from 1 d to 3–5 d after TBI induction and recovered by 5 d [41–43]. The current results lead to two potential conclusions. The first is that CHD might have beneficial effects for motor function after TBI that could be utilized in the chronic rehabilitation phase or in disease models associated with motor functional deficits. The second is that these effects might result from protective effects against damage to the cortex, which is the main processing center for limb movement. It could be used as further evidence for the effects of CHD against brain damage.

In addition to motor function, CHD also led to improved cognitive function as evaluated by the NOR test, which is a simple and representative tool to evaluate cognitive function in rodent models [44]. It is designed based on the natural preference of animals for a new object and helps evaluate cognitive and memory function with two different objects [44, 45]. The cognitive dysfunction in TBI is known to be obvious from 7-8 d after injury [46] and influenced by both hippocampal and cortical lesions, specifically lesions in the perirhinal cortex and medial temporal lobe [47]. The current result might be due to the reduction of damage to the parietal cortex and hippocampus. The current result could be also

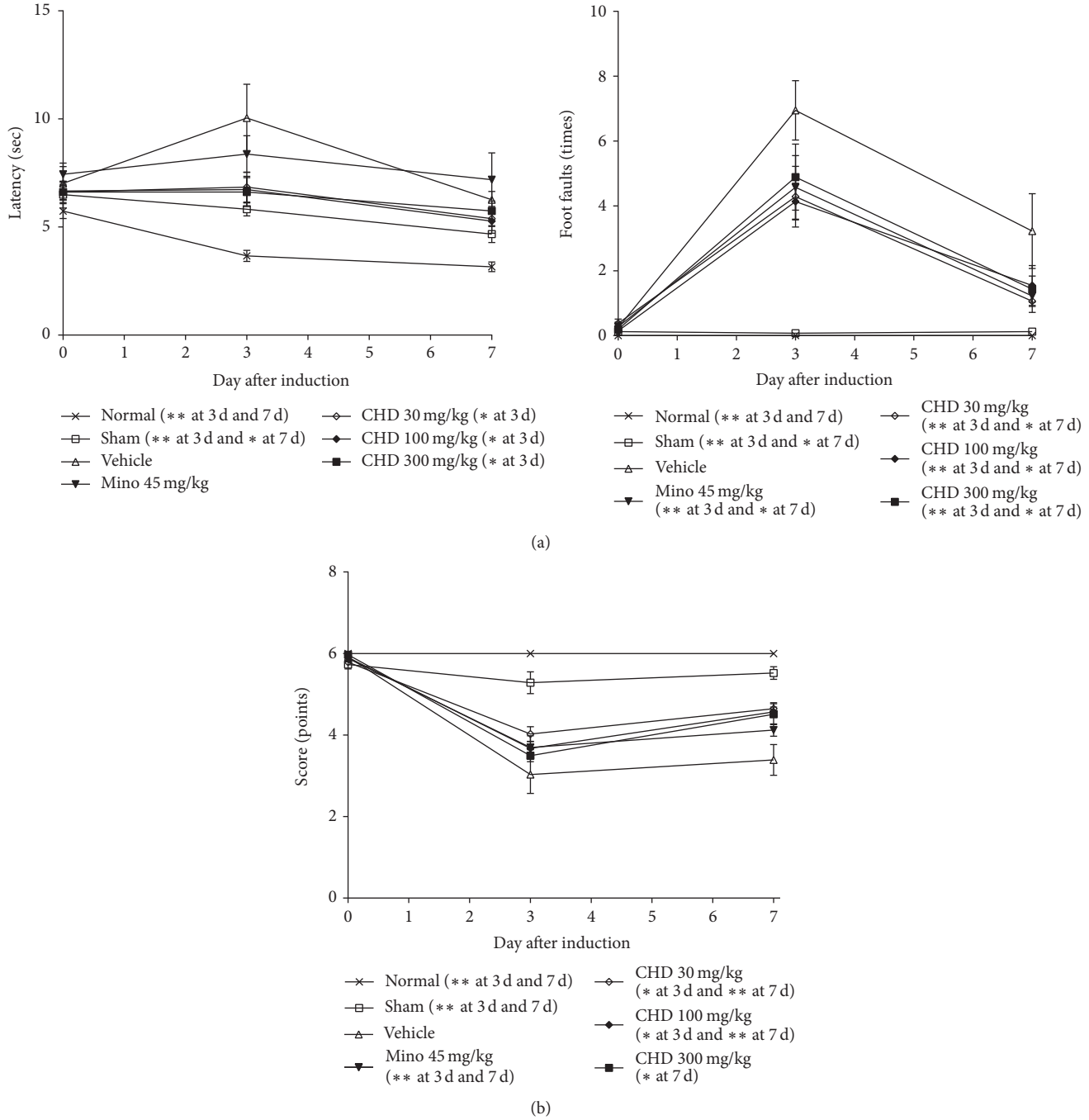

Figure 4: The effects of Chunghyul-Dan (CHD) on motor functional deficits in TBI mouse model using beam walking test and balance beam test. Graphs show the value at each time point (0 d (before) and 3 d and 7 d after TBI). (a) is latency (sec) and foot faults (times) of beam walking test and (b) is the score (points) of balance beam test. Values represent means ± SEM, $n = 8$. Mino is minocycline. ∗ represents statistical difference from vehicle-treated group at each time point ($^{*}P < 0.05$, $^{**}P < 0.01$).

supported by previous studies of CHD and its component herbs that demonstrated memory enhancing effects [48–52]. We could not identify the cause of the ineffectiveness of minocycline; it may be due to a statistical issue.

The positive control condition used in the current study, minocycline, has been reported to have protective effects against various brain injuries including TBI [47, 53, 54]. The dose of 45 mg/kg was chosen based on a previous report [47]. The dose of CHD in the current study was chosen with respect to the doses used clinically, which is typically 15 mg/kg a day (300 mg capsule, three times a day in a 60 kg human). The doses used in the current study, 30, 100, and 300 mg/kg (twice a day), correspond to 4, 13.3, and 40 times the typical human dosage. In general, the dose in mice could be considered to

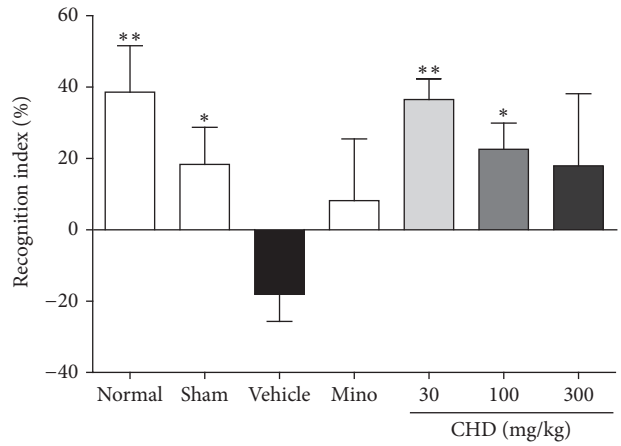

Figure 5: The effects of Chunghyul-Dan (CHD) on cognitive functional deficits in TBI mouse model using novel object recognition test. Graph shows the recognition index (%) of each group. Values represent means ± SEM, $n = 8$. Mino is minocycline-treated group. $*$ represents statistical difference from vehicle-treated group ($^{*}P < 0.05$, $^{**}P < 0.01$).

be approximately 12.3 times higher than that in a human according to the formula for dose translation [55]. Thus, 30–100 mg/kg might be the optimal dosage to use in murine studies.

5. Conclusion

CHD treatment could ameliorate brain injury and injury-related motor and cognitive functional deficits in a TBI mouse model at doses of 30 and 100 mg/kg. CHD could be a candidate agent of Korean medicine for patients with TBI, in addition to its current use in patients with stroke. Further studies are needed to assess the exact mechanisms of the effects in the acute-subacute phase and pharmacological activity during the chronic-convalescent phase of TBI.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Won-Woo Choi, Kyungjin Lee, and Beom-Joon Lee performed the experiments and drafted the manuscript. Seong-Uk Park and Jung-Mi Park designed the experimental protocol and commented on the manuscript. Chang-Nam Ko and Youngmin Bu conceived the project idea, designed the experimental protocol, and revised the manuscript. Won-Woo Choi and Kyungjin Lee contributed equally to current manuscript.

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References

[1] O. I. Schmidt, C. E. Heyde, W. Ertel, and P. F. Stahel, "Closed head injury—an inflammatory disease?" *Brain Research Reviews*, vol. 48, no. 2, pp. 388–399, 2005.

[2] J. V. Rosenfeld, A. I. Maas, P. Bragge, M. C. Morganti-Kossmann, G. T. Manley, and R. L. Gruen, "Early management of severe traumatic brain injury," *The Lancet*, vol. 380, no. 9847, pp. 1088–1098, 2012.

[3] D. J. Loane and A. I. Faden, "Neuroprotection for traumatic brain injury: translational challenges and emerging therapeutic strategies," *Trends in Pharmacological Sciences*, vol. 31, no. 12, pp. 596–604, 2010.

[4] N. Marklund and L. Hillered, "Animal modelling of traumatic brain injury in preclinical drug development: Where do we go from here?" *British Journal of Pharmacology*, vol. 164, no. 4, pp. 1207–1229, 2011.

[5] W. Wang, H. Li, J. Yu et al., "Protective Effects of Chinese Herbal Medicine Rhizoma drynariae in Rats After Traumatic Brain Injury and Identification of Active Compound," *Molecular Neurobiology*, vol. 53, no. 7, pp. 4809–4820, 2016.

[6] H. Quintard, T. Lorivel, C. Gandin, M. Lazdunski, and C. Heurteaux, "MLC901, a Traditional Chinese Medicine induces neuroprotective and neuroregenerative benefits after traumatic brain injury in rats," *Neuroscience*, vol. 277, pp. 72–86, 2014.

[7] K. J. Zhu and J. N. Sun, "Study on effect and mechanism of cinnabaris and realgar in promoting awake of endotoxin-induced brain injury rat applied with angong niuhuang wan," *China Journal of Chinese Materia Medica*, vol. 39, no. 20, pp. 4007–4012, 2014.

[8] S.-T. Yang, J.-W. Lin, B.-Y. Chiu, Y.-C. Hsu, C.-P. Chang, and C.-K. Chang, "Astragaloside improves outcomes of traumatic brain injury in rats by reducing microglia activation," *American Journal of Chinese Medicine*, vol. 42, no. 6, pp. 1357–1370, 2014.

[9] M.-C. Tsai, C.-P. Chang, S.-W. Peng et al., "Therapeutic efficacy of Neuro AiD (MLC 601), a traditional Chinese medicine, in experimental traumatic brain injury," *Journal of NeuroImmune Pharmacology*, vol. 10, no. 1, pp. 45–54, 2015.

[10] K. Cho, N. Ji, W. Jung, S. Park, S. Moon, and et al, "Chunghyul-dan for the prevention of stroke progression in silent brain infarction," *Journal of Korean Oriental Medicine*, vol. 26, no. 2, pp. 77–84, 2005.

[11] K. Cho, K. Noh, W. Jung et al., "A preliminary study on the inhibitory effect of Chunghyul-dan on stroke recurrence in patients with small vessel disease," *Neurological Research*, vol. 30, no. 6, pp. 655–658, 2008.

[12] Y.-S. Kim, E.-A. Jung, J. E. Shin, J. C. Chang, H. K. Yang et al., "Daio-Orengedokuto inhibits HMG-CoA reductase and pancreatic lipase," *Biological and Pharmaceutical Bulletin*, vol. 25, no. 11, pp. 1442–1445, 2002.

[13] K. H. Cho, H. S. Kang, W. S. Jung, S. U. Park, and S. K. Moon, "Efficacy and safety of Chunghyul-dan (Qingwie-dan) in patients with hypercholesterolemia," *The American Journal of Chinese Medicine*, vol. 33, no. 2, pp. 241–248, 2005.

[14] S. Park, W. Jung, S. Moon et al., "Chunghyuldan activates NOS mRNA expression and suppresses VCAM-1 mRNA expression in human endothelial cells," *Canadian Journal of Physiology and Pharmacology*, vol. 83, no. 12, pp. 1101–1108, 2005.

[15] K. H. Cho, Y. S. Kim, H. S. Bae, S. K. Moon, W. S. Jung et al., "Inhibitory effect of Chunghyuldan in prostaglandin E2 and nitric oxide biosynthesis of lipopolysaccharide-induced RAW 264.7 cells," *Biological and Pharmaceutical Bulletin*, vol. 27, no. 11, pp. 1810–1813, 2004.

[16] S. S. Wee, Y. W. Shin, E. A. Bae, and D. H. Kim, "Effect of chunghyuldan in chronic oxazolone-induced mouse dermatitis," *Biological and Pharmaceutical Bulletin*, vol. 28, no. 6, pp. 1079–1082, 2005.

[17] H. G. Kim, J.-Y. Kim, W.-W. Whang, and M. S. Oh, "Neuroprotective effect of chunghyuldan from amyloid beta oligomer induced neuroinflammation in vitro and in vivo," *Canadian Journal of Physiology and Pharmacology*, vol. 92, no. 6, pp. 429–437, 2014.

[18] C.-N. Ko, I.-S. Park, S.-U. Park et al., "Neuroprotective effect of Chunghyuldan (Qing Xue Dan) on hypoxia-reoxygenation induced damage of neuroblastoma 2a cell lines," *Chinese Journal of Integrative Medicine*, vol. 19, no. 12, pp. 940–944, 2013.

[19] S. P. Yun, W. S. Jung, S. U. Park et al., "Anti-hypertensive effect of *Chunghyul-dan* (*Qingxue-dan*) on stroke patients with essential hypertension," *American Journal of Chinese Medicine*, vol. 33, no. 3, pp. 357–364, 2005.

[20] S. U. Park, W. S. Jung, S. K. Moon et al., "Chunghyul-Dan (Qingxie-dan) improves arterial stiffness in patients with increased baPWV," *The American Journal of Chinese Medicine*, vol. 34, no. 4, pp. 553–563, 2006.

[21] G. Onyszchuk, B. Al-Hafez, Y.-Y. He, M. Bilgen, N. E. J. Berman, and W. M. Brooks, "A mouse model of sensorimotor controlled cortical impact: Characterization using longitudinal magnetic resonance imaging, behavioral assessments and histology," *Journal of Neuroscience Methods*, vol. 160, no. 2, pp. 187–196, 2007.

[22] S. Homsi, F. Federico, N. Croci et al., "Minocycline effects on cerebral edema: Relations with inflammatory and oxidative stress markers following traumatic brain injury in mice," *Brain Research*, vol. 1291, pp. 122–132, 2009.

[23] L. B. Goldstein and J. N. Davis, "Beam-walking in rats: studies towards developing an animal model of functional recovery after brain injury," *Journal of Neuroscience Methods*, vol. 31, no. 2, pp. 101–107, 1990.

[24] C. Messier, "Object recognition in mice: Improvement of memory by glucose," *Neurobiology of Learning and Memory*, vol. 67, no. 2, pp. 172–175, 1997.

[25] C. B. Matthew, I. V. Sils, and A. M. Bastille, "Tissue-specific extravasation of albumin-bound Evans blue in hypothermic and rewarmed rats," *Canadian Journal of Physiology and Pharmacology*, vol. 80, no. 3, pp. 233–243, 2002.

[26] Y. Xiong, A. Mahmood, and M. Chopp, "Animal models of traumatic brain injury," *Nature Reviews Neuroscience*, vol. 14, no. 2, pp. 128–142, 2013.

[27] H. G. Kim, M. S. Ju, D. H. Kim, J. Hong, S. H. Cho et al., "Protective effects of Chunghyuldan against ROS-mediated neuronal cell death in models of Parkinson's disease," *Basic and Clinical Pharmacology and Toxicology*, vol. 107, no. 6, pp. 958–964, 2010.

[28] G. Kim, J. Kim, S. Chung, S. Cho, M. Oh et al., "The protective effect of Chunghyul-dan(Qingxuedan) against 6-hydroxydopamine Induced neurotoxicity," *Journal of Oriental Neuropsychiatry*, vol. 20, no. 1, pp. 21–42, 2009.

[29] N. Oshima, Y. Narukawa, N. Hada, and F. Kiuchi, "Quantitative analysis of anti-inflammatory activity of orengedokuto: importance of combination of flavonoids in inhibition of PGE2 production in mouse macrophage-like cell line J774.1," *Journal of Natural Medicines*, vol. 67, no. 2, pp. 281–288, 2013.

[30] X. Y. Kong, W. Kong, G. X. Miao et al., "Pretreatment with scutellaria baicalensis stem-leaf total flavonoid protects against cerebral ischemia/ reperfusion injury in hippocampal neurons," *Neural Regeneration Research*, vol. 9, no. 23, pp. 2066–2073, 2014.

[31] Y. Shang, G. Miao, H. Zhao et al., "Mechanisms underlying attenuation of apoptosis of cortical neurons in the hypoxic brain by flavonoids from the stems and leaves of *Scutellaria baicalensis* Georgi," *Neural Regeneration Research*, vol. 9, no. 17, pp. 1592–1598, 2014.

[32] Q. Zhang, Z. Qian, L. Pan, H. Li, and H. Zhu, "Hypoxia-inducible factor 1 mediates the anti-apoptosis of berberine in neurons during hypoxia/ischemia," *Acta Physiologica Hungarica*, vol. 99, no. 3, pp. 311–323, 2012.

[33] R. H. Hughes, V. A. Silva, I. Ahmed, D. I. Shreiber, and B. Morrison III, "Neuroprotection by genipin against reactive oxygen and reactive nitrogen species-mediated injury in organotypic hippocampal slice cultures," *Brain Research*, vol. 1543, pp. 308–314, 2014.

[34] Y.-K. Park, Y. S. Chung, Y. S. Kim, O.-Y. Kwon, and T. H. Joh, "Inhibition of gene expression and production of iNOS and TNF-α in LPS-stimulated microglia by methanol extract of Phellodendri cortex," *International Immunopharmacology*, vol. 7, no. 7, pp. 955–962, 2007.

[35] F. Li, W. Li, X. Li et al., "Geniposide attenuates inflammatory response by suppressing P2Y14 receptor and downstream ERK1/2 signaling pathway in oxygen and glucose deprivation-induced brain microvascular endothelial cells," *Journal of Ethnopharmacology*, vol. 185, pp. 77–86, 2016.

[36] Y. Chen, Y. Zhang, L. Li, and C. Hölscher, "Neuroprotective effects of geniposide in the MPTP mouse model of Parkinson's disease," *European Journal of Pharmacology*, vol. 768, pp. 21–27, 2015.

[37] K. Lu, C. Zhang, W. Wu, M. Zhou, Y. Tang et al., "Rhubarb extract has a protective role against radiation-induced brain injury and neuronal cell apoptosis," *Molecular Medicine Reports*, vol. 12, no. 2, pp. 2689–2694, 2015.

[38] R. J. Hamm, D. M. White-Gbadebo, B. G. Lyeth, L. W. Jenkins, and R. L. Hayes, "The effect of age on motor and cognitive deficits after traumatic brain injury in rats," *Neurosurgery*, vol. 31, no. 6, pp. 1072–1078, 1992.

[39] B. G. Lyeth, Ji Yao Jiang, and S. Liu, "Behavioral protection by moderate hypothermia initiated after experimental traumatic brain injury," *Journal of Neurotrauma*, vol. 10, no. 1, pp. 57–64, 1993.

[40] N. I. Wood, B. V. Sopesen, J. C. Roberts et al., "Motor dysfunction in a photothrombotic focal ischaemia model," *Behavioural Brain Research*, vol. 78, no. 2, pp. 113–120, 1996.

[41] R. H. Singleton, H. Q. Yan, W. Fellows-Mayle, and C. E. Dixon, "Resveratrol attenuates behavioral impairments and reduces cortical and hippocampal loss in a rat controlled cortical impact model of traumatic brain injury," *Journal of Neurotrauma*, vol. 27, no. 6, pp. 1091–1099, 2010.

[42] J. Tsenter, L. Beni-Adani, Y. Assaf, A. G. Alexandrovich, V. Trembovler, and E. Shohami, "Dynamic changes in the recovery after traumatic brain injury in mice: Effect of injury severity on T2-weighted MRI abnormalities, and motor and cognitive

functions," *Journal of Neurotrauma*, vol. 25, no. 4, pp. 324–333, 2008.

[43] S. S. Shin and C. E. Dixon, "Alterations in cholinergic pathways and therapeutic strategies targeting cholinergic system after traumatic brain injury," *Journal of Neurotrauma*, vol. 32, no. 19, pp. 1429–1440, 2015.

[44] A. Ennaceur and J. Delacour, "A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data," *Behavioural Brain Research*, vol. 31, no. 1, pp. 47–59, 1988.

[45] M. Antunes and G. Biala, "The novel object recognition memory: neurobiology, test procedure, and its modifications," *Cognitive Processing*, vol. 13, no. 2, pp. 93–110, 2012.

[46] R. Baratz, D. Tweedie, J.-Y. Wang et al., "Transiently lowering tumor necrosis factor-alpha synthesis ameliorates neuronal cell loss and cognitive impairments induced by minimal traumatic brain injury in mice," *Journal of Neuroinflammation*, vol. 12, article 45, 2015.

[47] N. Bye, M. D. Habgood, J. K. Callaway et al., "Transient neuroprotection by minocycline following traumatic brain injury is associated with attenuated microglial activation but no changes in cell apoptosis or neutrophil infiltration," *Experimental Neurology*, vol. 204, no. 1, pp. 220–233, 2007.

[48] J. G. Choi, M. Moon, H. G. Kim et al., "Gami-Chunghyuldan ameliorates memory impairment and neurodegeneration induced by intrahippocampal Aβ1-42 oligomer injection," *Neurobiology of Learning and Memory*, vol. 96, no. 2, pp. 306–314, 2011.

[49] Y. Z. Shang, H. Zhang, J. J. Cheng, H. Miao, Y. P. Liu et al., "Flavonoids from scutellaria baicalensis georgi are effective to treat cerebral ischemia/reperfusion," *Neural Regeneration Research*, vol. 8, no. 6, pp. 514–522, 2013.

[50] F. Zhu and C. Qian, "Berberine chloride can ameliorate the spatial memory impairment and increase the expression of interleukin-1beta and inducible nitric oxide synthase in the rat model of Alzheimer's disease," *BMC Neuroscience*, vol. 7, no. 1, 2006.

[51] B. Lee, B. Sur, I. Shim, H. Lee, and D. H. Hahm, "*Phellodendron amurense* and its major alkaloid compound, berberine ameliorates scopolamine-induced neuronal impairment and memory dysfunction in rats," *The Korean Journal of Physiology and Pharmacology*, vol. 16, no. 2, pp. 79–89, 2012.

[52] Y. Nam and D. Lee, "Ameliorating effect of Zhizi (Fructus Gardeniae) extract and its glycosides on scopolamine-induced memory impairment," *Journal of Traditional Chinese Medicine*, vol. 33, no. 2, pp. 223–227, 2013.

[53] K. L. Arvin, B. H. Han, Y. Du, S.-Z. Lin, S. M. Paul, and D. M. Holtzman, "Minocycline markedly protects the neonatal brain against hypoxic-ischemic injury," *Annals of Neurology*, vol. 52, no. 1, pp. 54–61, 2002.

[54] Y. Du, Z. Ma, S. Lin et al., "Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 25, pp. 14669–14674, 2001.

[55] S. Reagan-Shaw, M. Nihal, and N. Ahmad, "Dose translation from animal to human studies revisited," *The FASEB Journal*, vol. 22, no. 3, pp. 659–661, 2008.

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Research Article

Changes in Body Water Caused by Sleep Deprivation in Taeeum and Soyang Types in Sasang Medicine: Prospective Intervention Study

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Background. There is a negative relationship between sleep deprivation and health. However, no study has investigated the effect of sleep deprivation on individuals with different body composition. The aim of this study was to determine the differential effect of sleep deprivation in individuals with different body compositions (fluid) according to Soyang type (SY) and Taeeum type (TE). *Methods*. Sixty-two cognitively normal, middle-aged people with normal sleep patterns were recruited from the local population. The duration of participants' sleep was restricted to 4 h/day during the intervention phase. To examine the physiological changes brought on by sleep deprivation and recovery, 10 ml of venous blood was obtained. *Results*. Total Body Water (TBW) and Extracellular Water (ECW) were significantly different between the groups in the intervention phase. Physiological parameters also varied from the beginning of the resting phase to the end of the experiment. Potassium levels changed more in SY than TE individuals. *Conclusion*. Participants responded differently to the same amount of sleep deprivation depending on their Sasang constitution types. This study indicated that SY individuals were more sensitive to sleep deprivation and were slower to recover from the effects of sleep deprivation than TE individuals.

1. Background

Sufficient sleep is essential for health and wellbeing. Sleep is closely associated with the regulation of energy balance and metabolism [1], and several studies have shown a relationship between sleep and health [2–5]. Sleep disturbances lead to physical changes; for instance, 48 h sleep deprivation has been reported to increase plasma levels of thyroid hormones [6]. In addition, participants who had been sleep-deprived for 72 h demonstrated increased levels of urea, suggesting increased protein catabolism and gluconeogenesis [7]. Furthermore, disturbed sleep can be lead to disease states. The quantity and quality of sleep has been associated with a greater risk of developing type 2 diabetes [8]. Short sleep duration has also been associated with increased blood pressure and an increased risk of hypertension [9]. Sleeping for less than 6 h or more than 7 h a night is associated with an increased risk of death [2]. However, no study has yet examined the variation in these associations between individuals with different body types.

Sasang constitutional medicine (SCM), developed by Lee Je-ma, is a type of Korean personalized traditional medicine. SCM is widely used for clinical diagnosis and treatment of disease in Korea. SCM classifies people into four types:

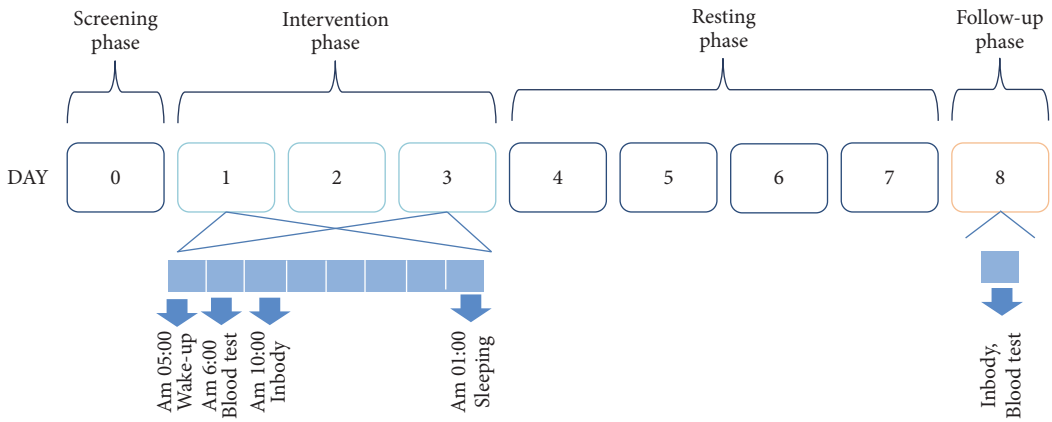

Figure 1: Study design.

Taeyang type (TY), Soyang type (SY), Taeeum type (TE), and Soum type (SE) [10]. The Sasang constitution (SC) types have different pathophysiological susceptibilities and have different risks for various diseases [11]. Previous studies have revealed that SC types could be considered risk factors for certain chronic diseases, including diabetes mellitus (DM), hypertension, abdominal obesity, metabolic syndrome, functional dyspepsia, obstructive sleep apnea, and subclinical hypothyroidism [12–15].

SC types are considered to be crucial in predisease and disease stages from a preventive medicine point of view [16]. Therefore, it is also relevant to determine whether SC type is a clinically important risk factor in the predisease stage. However, only one study has found an association between predisease state (pre-HTN) and SC type [11]. Thus, the current study aimed to determine differences in body composition (fluid) in individuals with different SC types after sleep deprivation.

2. Methods

2.1. Study Design and Intervention. This was a prospective interventional study. The protocol of this study and the consent forms given to participants were approved by the University of KyungHee Institutional Review Board (KHNMCOH 2015-08-002-002). All participants gave written informed consent

As shown in Figure 1, 2 weeks to 1 month after the screening examination, participants registered at the Clinical Research Unit (Sasang Constitution in Kyung Hee University Korean Medicine Hospital in Gangdong (KHUKMHG)) and began the 3-day, 2-night intervention phase of the study (days 1, 2, and 3). During the intervention phase, participants were restricted to 4 h of sleep time and fed a controlled diet based on their weight-maintenance energy by trained research nurses. Participants were asked to avoid caffeine and alcohol for 24 h before the intervention phase. After undergoing sleep restriction (01:00 to 05:00), physiological parameters, including blood test and Inbody test, were assessed. After the intervention phase, participants were allowed to rest for 4 days and 3 nights (days 4, 5, 6, and 7). During the resting phase, participants were allowed to sleep at will. After the resting phase, participants once again visited the Clinical Research Unit and underwent follow-up tests in the follow-up phase (day 8).

2.2. Subjects. In total, 80 participants (aged 35–45 years) were screened first by interview and then during visits to our clinic. Finally, 62 subjects were included in this study. All subjects met the following criteria: those who had slept 7-8 h per night over the previous week and who did not have sleep disorders, as reflected by a Pittsburgh sleep quality index (PSQI) score under 5 within the last month. Participants who did Chalder Fatigue Scale (CFS) score over 19 within the last month or body pain score as assessed by the Visual Analog Scale (VAS) score 40 mm at any time within the previous month were excluded. Pregnant women or those who has a history of psychiatric or neurological disorders 6 months prior to the study were also excluded (Figure 2).

2.3. Data Collection. For the blood test, 10 ml of venous blood was obtained from each subject on four occasions: days 1 and 3 (intervention phase) and day 8 (follow-up phase). During the study, blood was sampled every 30 min starting at 06:00. Blood samples were obtained by peripheral venous puncture, immediately centrifuged at 3000g for 10 min and stored at −80°C until required for analysis. Standard methods were used to measure serum electrolyte levels, such as sodium, potassium, calcium, phosphate, creatinine, urea, and uric acid, with an ADVIA 2120i (Siemens, USA).

Participants' body composition was determined with segmental bioelectric impedance using electrodes, according to the manufacturer's instructions (Inbody 770, Biospace Co. Ltd, Seoul, Korea). Daily at 10:00, a pair of electrodes was placed on the surfaces of the thumb, palm, and fingers of the hand, and on the ball of the foot. Microprocessor-controlled switches and an impedance analyzer were used to measure segmental resistance at four frequencies (5, 50, 250, and 500 kHz) Thus, a set of 20 segmental resistances was obtained from each individual. With these data, Total Body Water (TBW), Extracellular Water (ECW), and Intracellular Water (ICW) were calculated from the sum of each segment,

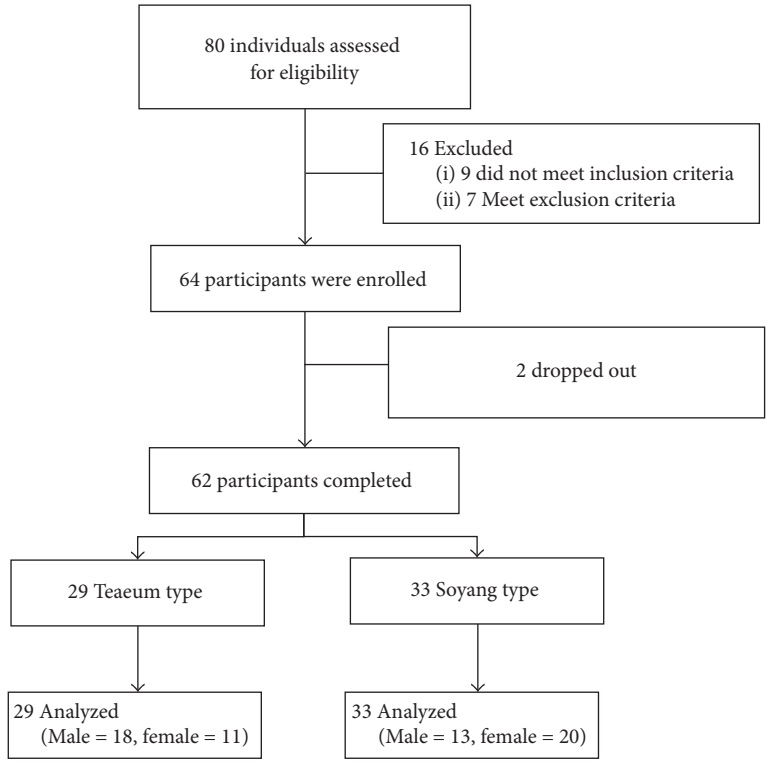

Figure 2: CONSORT flow diagram.

using the equations provided in the BIA software [17–19]. The subjects' height and weight were measured and rounded to the nearest hundredth, and body mass index (BMI) was indirectly calculated from the height and weight of each individual.

2.4. Diagnosis of SC Type. Diagnosis of the participants' SC types was based on a medical chart review conducted by licensed medical specialists in Korean Sasang typology who had been in clinical practice for 10 years or more. They classified the subjects' SC types based on temperament, body shape, appearance, voice, and pathophysiological characteristics of each individual.

2.5. Statistical Analysis. Data obtained before and after the intervention were analyzed using descriptive statistics (Chi-square tests, Mann–Whitney U tests, and t-tests) based on SPSS software version 17. The level of statistical significance was set at $p < 0.05$. A Kolmogorov-Smirnov test was used to determine whether the data were normally distributed ($p = 0.9$), and the homogeneity of variances was determined ($p = 0.21$).

3. Results

3.1. Subjects Characteristics. Table 1 shows the general and anthropometric characteristics of the subjects by SC type. The numbers of participants with TE and SY constitutional types were 18 men and 11 women ($n = 29$) and 13 men and 20 women ($n = 33$), respectively. The mean ages of the participants in the TE group and the SY group were 39.86 years and 39.56 years, respectively. Independent t-tests revealed that there were no significant differences between the groups in terms of age and height. A chi-square test showed no significant difference between the groups in terms of sex distribution. Overall, both groups had similar background characteristics. Statistical tests also showed no differences between the two groups except for weight and BMI.

3.2. Body Composition Changes. Table 2 presents a summary of the body composition of participants measured by Inbody. There was no significant difference between the two groups in terms of TBW, ECW, or ICW during the intervention phase (days 1, 2, and 3) or in the follow-up phase (day 8). However, TBW and ECW varied significantly during the intervention phase (difference between day 1 and day 3, $p < 0.05$), and also during the follow-up phase (difference between day 3 and day 8, $p < 0.05$). No significant difference was found between the two groups in terms of TBW and ECW in a cross-sectional comparison. However, there was a significant difference between TBW and ECW between groups ($p < 0.05$). Variation in TBW during the intervention phase (day 1 to day 3) was 0.14 ± 0.77 in the TE group and 0.51 ± 0.86 in the SY group. Variation in TBW during the resting phase (day 3–8) was 0.34 ± 0.93 and 1.15 ± 0.64 in the two groups, respectively. Variation in ECW during the intervention phase was 0.02 ± 0.28 and 0.18 ± 0.33 in the TE and SY groups, respectively, while variation in ECW during the resting phase was 0.09 ± 0.41 and 0.42 ± 0.31 in the two groups, respectively.

Table 1: Overview of baseline characteristics.

Variable	Taeeum type ($N = 29$) Mean (SD)	Soyang type ($N = 33$) Mean (SD)	p value*
Gender, N	Male-18 Female-11	Male-13 Female-20	0.52
Age (y)	39.86 ± 2.48	39.56 ± 2.83	0.91
Height (cm)	169.84 ± 9.06	166.64 ± 7.43	0.13
Weight (kg)	67.00 ± 10.12	60.02 ± 8.97	0.05†
BMI (kg/m^2)	23.04 ± 1.82	21.49 ± 2.15	0.03†
PSQI	3.95 ± 1.02	4.01 ± 1.32	0.45

SD: standard deviation, N: number, y: year, BMI: body mass index, and PSQI: Pittsburg Sleep Quality Index.
* p values are addressed by Student's t-test, χ^2-test between Taeeum type and Soyang type ($\alpha = 0.05$).
† $p < 0.05$ from Student's t-test.

Table 2: The difference of body water between Taeeum type and Soyang type.

Variable	Taeeum type ($N = 29$) Mean ± SD	Soyang type ($N = 33$) Mean ± SD	p value*
ICW (L)			
D3-D1	0.13 ± 0.49	0.37 ± 0.56	0.08
D3-D8	0.23 ± 0.54	0.59 ± 0.88	0.06
ECW (L)			
D3-D1	0.02 ± 0.28	0.18 ± 0.33	0.05†
D3-D8	0.09 ± 0.41	0.42 ± 0.31	0.00†
TBW (L)			
D3-D1	0.14 ± 0.77	0.51 ± 0.86	0.05†
D3-D8	0.34 ± 0.93	1.15 ± 0.64	0.00†

N: number, SD: standard deviation, ICW: intracellular water, ECW: extracellular water, TBW: total body water, D1: day 1, D3: day 3, D8: day 8, D3-D1: differences in the intervention phase, and D3-D8: differences in the resting phase.
* p values are addressed by Student's t-test ($\alpha = 0.05$).
† $p < 0.05$ from Student's t-test.

3.3. Blood Analysis Changes. Table 3 summarizes the changes in the results of blood analyses. There were no differences in serum electrolyte and albumin levels, or in liver and renal function test results. Electrolyte levels did not change significantly between the intervention phase and the follow-up phase (days 1, 3, and 8). However, potassium level variation showed a constant trend: the variation in potassium levels in the intervention phase (day 3 versus day 1) was 0.09 ± 1.02 and 0.23 ± 0.41 in the TE and SY groups, respectively (Table 3). There was no significant difference between the two groups in terms of liver function and renal function test results in the intervention and follow-up phases (days 1, 3, and 8). Both groups exhibited levels of AST, ALT, BUN, and creatinine that were within normal ranges.

4. Discussion

This study focused on SC types as risk factors for the effects of sleep deprivation. There were no significant differences in terms of TBW, ECW, and ICW in the SY and TE groups before the intervention phase. Variation in TBW and ECW was higher in SY individuals than in TE individuals after 3 days of sleep deprivation, whereas variation was lower in TE individuals after 4 days of the resting phase. This indicated that SY-type individuals are more sensitive to sleep deprivation and are slower to recover from physiological changes brought about by sleep deprivation.

TBW was distributed between two compartments, the ECW and the ICW. The composition of ECW is regulated by various mechanisms, but especially by the kidneys [20]. Liver and renal function tests were not significantly different between the two groups. However, potassium levels were different between the two groups. This trend supports the hypothesis that sleep deprivation may influence sodium-potassium pumps. Previous studies have suggested that sleep deprivation increases sodium-potassium pump activity and that this increase may be mediated by the action of norepinephrine on either alpha-1/-2 receptors or increased turnover of sodium-potassium pump molecules [21, 22]. Furthermore, this increased activity of the sodium-potassium pump induced oxidative stress [23, 24]. Therefore, reactive oxygen species (ROS) may be produced during the sleep deprivation process, as suggested by other studies [25, 26].

Metabolic syndrome, a collection of cardiometabolic risk factors that includes obesity, insulin resistance, hypertension, and dyslipidemia [27], is often characterized by oxidative

Table 3: The difference of electrolyte, liver, and renal functions between Taeeum type and Soyang type.

Variable	Taeeum type ($N = 29$) Mean ± SD	Soyang type ($N = 33$) Mean ± SD	p value*
K (mmol/L)			
D3-D1	0.09 ± 1.02	0.23 ± 0.41	0.05†
D3-D8	1.02 ± 7.04	−0.39 ± 0.54	0.25
Na (mmol/L)			
D3-D1	0.13 ± 1.42	0.00 ± 1.56	0.72
D3-D8	0.27 ± 1.38	0.42 ± 1.63	0.70
Ca (mg/dL)			
D3-D1	−2.31 ± 0.17	−0.15 ± 0.33	0.30
D3-D8	−0.035 ± 0.30	−0.30 ± 0.32	0.51
Alb (g/dL)			
D3-D1	−0.20 ± 0.15	−0.18 ± 0.20	0.65
D3-D8	−0.34 ± 0.22	−0.37 ± 0.19	0.31
BUN (mg/dL)			
D3-D1	−1.33 ± 2.62	−2.19 ± 2.37	0.18
D3-D8	−1.43 ± 3.63	−2.05 ± 2.72	0.45
Cr (mg/dL)			
D3-D1	0.01 ± 0.68	0.00 ± 0.04	0.20
D3-D8	0.00 ± 0.06	−0.01 ± 0.05	0.21
AST (U/L)			
D3-D1	−0.62 ± 3.83	−0.52 ± 5.67	0.93
D3-D8	0.03 ± 4.06	−1.06 ± 6.87	0.46
ALT (U/L)			
D3-D1	−2.34 ± 4.84	−0.18 ± 8.51	0.23
D3-D8	−1.31 ± 4.89	−1.42 ± 8.73	0.95

N: number, SD: standard deviation, K: potassium, Na: natrium, Ca: calcium, Alb: albumin, BUN: blood urea nitrogen, Cr: creatinine, AST: aspartate aminotransferase, ALT: alanine aminotransferase, D1: day 1, D3: day 3, D8: day 8, D3-D1: differences in the intervention phase, and D3-D8: differences in the resting phase.

* p values are addressed by Student's t-test ($\alpha = 0.05$).

† $p < 0.05$ from Student's t-test.

stress, a condition involving an imbalance between the production and inactivation of ROS. Therefore in the present study, although the intervention phase involved only a 3-day, 2-night period, SY individuals may be more susceptible to metabolic syndrome. In addition, the effect of hormones (aldosterone, cortisol, etc.) and the interaction between cardiac output and peripheral arterial resistance may be involved in this difference in individual responses [28–30]. In future, the association between metabolic syndrome, various hormonal changes and cardiovascular diseases, and sleep deprivation and cell volume regulation should be investigated.

According to Sasang constitutional medicine, sleep deprivation is one of a list of symptoms [31] that are used to identify an individual's constitution and a number of constitutional diseases [32]. In fact, a previous study of 1229 patients who attended department of Sasang constitutional medicine for their first medical examination showed that SY individuals typically experienced poor sleep quality and quantity [33].

The main strength of this study is the restriction of sleep deprivation to clinical research conditions. However, there are some limitations to this study. First, the intervention phase was only 3 days, which is too short a period to elicit changes in the body. Second, constitution types were not evenly distributed across study participants but were biased toward the TE and SY. Therefore, the present findings can not be generalized to So Eum type (SE). Future studies should include a large number of participants and should be performed across institutes using a prospective clinical research design, in order to derive a plausible model for predicting disease caused by lack of sleep.

5. Conclusion

This study showed that TBW, ECW, and ICW are not significantly different between the SY and TE in a cross-sectional comparison. However, variations in TBW and ECW were greater in SY individuals and were higher than in

TE-type individuals after 3 days of sleep deprivation, and SY-type individuals recovered more slowly than TE-type individuals did during the 4-day resting period after sleep deprivation. Liver and renal function parameters and blood sodium, potassium, and albumin concentrations were not significantly different between the two groups, although potassium levels were altered differentially across the two groups. Thus, individuals respond differently to the same amount of sleep deprivation according to SC types.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] J. M. Siegel, "Clues to the functions of mammalian sleep," *Nature*, vol. 437, no. 7063, pp. 1264–1271, 2005.

[2] S. R. Patel, N. T. Ayas, M. R. Malhotra et al., "A prospective study of sleep duration and mortality risk in women," *Sleep*, vol. 27, no. 3, pp. 440–444, 2004.

[3] C. A. Everson and A. Szabo, "Recurrent restriction of sleep and inadequate recuperation induce both adaptive changes and pathological outcomes," *American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 297, no. 5, pp. R1430–R1440, 2009.

[4] D. F. Kripke, L. Garfinkel, D. L. Wingard, M. R. Klauber, and M. R. Marler, "Mortality associated with sleep duration and insomnia," *Archives of General Psychiatry*, vol. 59, no. 2, pp. 131–136, 2002.

[5] A. Tamakoshi and Y. Ohno, "Self-reported sleep duration as a predictor of all-cause mortality: results from the JACC Study, Japan," *Sleep*, vol. 27, no. 1, pp. 51–54, 2004.

[6] H.-W. Feng, T. Jiang, H.-P. Zhang et al., "Comparisons of thyroid hormone, intelligence, attention, and quality of life in children with obstructive sleep apnea hypopnea syndrome before and after endoscopic adenoidectomy," *BioMed Research International*, vol. 2015, Article ID 523716, 6 pages, 2015.

[7] G. J. Kant, S. G. Genser, D. R. Thorne, J. L. Pfalser, and E. H. Mougey, "Effects of 72 hour sleep deprivation on urinary cortisol and indices of metabolism," *Sleep*, vol. 7, no. 2, pp. 142–146, 1984.

[8] F. P. Cappuccio, L. D'Elia, P. Strazzullo, and M. A. Miller, "Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis," *Diabetes Care*, vol. 33, no. 2, pp. 414–420, 2010.

[9] S. Larcher, P. Benhamou, J. Pépin, and A. Borel, "Sleep habits and diabetes," *Diabetes & Metabolism*, vol. 41, no. 4, pp. 263–271, 2015.

[10] Y. J. Kim, Y. C. Ahn, and C. G. Son, "Sasang constitution affects the prevalence of functional dyspepsia," *BMC Complementary and Alternative Medicine*, vol. 15, no. 1, pp. 150–154, 2015.

[11] E. Jang, Y. Baek, Y. Kim, K. Park, and S. Lee, "Sasang constitution may act as a risk factor for prehypertension," *BMC Complementary and Alternative Medicine*, vol. 15, no. 1, article no. 231, 2015.

[12] T.-G. Lee, B. Koh, and S. Lee, "Sasang constitution as a risk factor for diabetes mellitus: a cross-sectional study," *Evidence-Based Complementary and Alternative Medicine*, vol. 6, no. 1, pp. 99–103, 2009.

[13] K. Choi, J. Lee, J. Yoo, E. Lee, B. Koh, and J. Lee, "Sasang constitutional types can act as a risk factor for insulin resistance," *Diabetes Research and Clinical Practice*, vol. 91, no. 3, pp. e57–e60, 2011.

[14] E. Jang, Y. Baek, K. Park, and S. Lee, "Could the Sasang constitution itself be a risk factor of abdominal obesity?" *BMC Complementary and Alternative Medicine*, vol. 13, pp. 72–77, 2013.

[15] K. H. Song, S.-G. Yu, and J. Y. Kim, "Prevalence of metabolic syndrome according to sasang constitutional medicine in Korean subjects," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 646794, 8 pages, 2012.

[16] J.-H. Do, E. Jang, B. Ku, J.-S. Jang, H. Kim, and J. Y. Kim, "Development of an integrated Sasang constitution diagnosis method using face, body shape, voice, and questionnaire information," *BMC Complementary and Alternative Medicine*, vol. 12, pp. 85–93, 2012.

[17] M. Okamoto, M. Fukui, A. Kurusu et al., "Usefulness of a body composition analyzer, inbody 2.0, in chronic hemodialysis patients," *Kaohsiung Journal of Medical Sciences*, vol. 22, no. 5, pp. 207–210, 2006.

[18] K. Cha, G. M. Chertow, J. Gonzalez, J. M. Lazarus, and D. W. Wilmore, "Multifrequency bioelectrical impedance estimates the distribution of body water," *Journal of Applied Physiology*, vol. 79, no. 4, pp. 1316–1319, 1995.

[19] S. W. Lee, J. H. Song, G. A. Kim, K. J. Lee, and M.-J. Kim, "Assessment of total body water from anthropometry-based equations using bioelectrical impedance as reference in Korean adult control and haemodialysis subjects," *Nephrology Dialysis Transplantation*, vol. 16, no. 1, pp. 91–97, 2001.

[20] H. Y. Choi and S. K. Ha, "Potassium balances in maintenance hemodialysis," *Electrolyte and Blood Pressure*, vol. 11, no. 1, pp. 9–16, 2013.

[21] S. Gulyani and B. N. Mallick, "Possible mechanism of rapid eye movement sleep deprivation induced increase in N-K ATPase activity," *Neuroscience*, vol. 64, no. 1, pp. 255–260, 1995.

[22] S. Majumdar, M. Faisal, V. Madan, and B. N. Mallick, "Increased turnover of Na-K ATPase molecules in rat brain after rapid eye movement sleep deprivation," *Journal of Neuroscience Research*, vol. 73, no. 6, pp. 870–875, 2003.

[23] C.-C. Liu, N. A. S. Fry, E. J. Hamilton et al., "Redox-dependent regulation of the Na+-K+ pump: new twists to an old target for treatment of heart failure," *Journal of Molecular and Cellular Cardiology*, vol. 61, pp. 94–101, 2013.

[24] G. A. Figtree, G. Keyvan Karimi, C. Liu, and H. H. Rasmussen, "Oxidative regulation of the Na+-K+ pump in the cardiovascular system," *Free Radical Biology and Medicine*, vol. 53, no. 12, pp. 2263–2268, 2012.

[25] G. Villafuerte, A. Miguel-Puga, E. Murillo Rodríguez, S. Machado, E. Manjarrez, and O. Arias-Carrión, "Sleep deprivation and oxidative stress in animal models: a systematic review," *Oxidative Medicine and Cellular Longevity*, vol. 2015, Article ID 234952, 15 pages, 2015.

[26] J. Noguti, M. L. Andersen, C. Cirelli, and D. A. Ribeiro, "Oxidative stress, cancer, and sleep deprivation: is there a logical link in this association?" *Sleep and Breathing*, vol. 17, no. 3, pp. 905–910, 2013.

[27] C. K. Roberts and K. K. Sindhu, "Oxidative stress and metabolic syndrome," *Life Sciences*, vol. 84, no. 21-22, pp. 705–712, 2009.

[28] J. J. Cohen, H. N. Hulter, N. Smithline, J. C. Melby, and W. B. Schwartz, "The critical role of the adrenal gland in the renal regulation of acid base equilibrium during chronic hypotonic expansion: evidence that chronic hyponatremia is

a potent stimulus to aldosterone secretion," *Journal of Clinical Investigation*, vol. 58, no. 5, pp. 1201–1208, 1976.

[29] R. L. Howard and R. W. Schrier, "A unifying hypothesis of sodium and water regulation in health and disease," *Hormone Research in Paediatrics*, vol. 34, no. 3-4, pp. 118–123, 1990.

[30] S.-H. Lin and M. L. Halperin, "Hypokalemia: a practical approach to diagnosis and its genetic basis," *Current Medicinal Chemistry*, vol. 14, no. 14, pp. 1551–1565, 2007.

[31] I. B. Song and S. M. KIM, "A Study of ordinary symtoms in the Dongyi Soose Bowon Sasang Chobongyun and DongmuYugo," *Journal of Sasang Constitute Med*, vol. 12, no. 2, pp. 67–77, 2000.

[32] J. Y. Choi, Y. S. Choi Lee Jr., and S. S. Park, "A study on the clinical features of oridinary sleeping patterns based on the sasang constitution, using the logistic regression," *Journal of Korean Oriental Med*, vol. 25, no. 4, pp. 171–179, 2004.

[33] J. J. Kim, Y. S. Lee, and S. S. Park, "A clinical study on the ordinary sleeping patterns of soyangin," *Journal of Oriental Neuropsychiatry*, vol. 16, no. 2, pp. 65–72, 2005.

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Research Article

The Current Status of Quality of Reporting in Acupuncture Treatment Case Reports: An Analysis of the Core Journal in Korea

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Objectives. The present study aimed to evaluate the overall quality of case reports concerning acupuncture treatment in Korea. *Methods*. We selected a representative Korean journal and retrieved eligible case reports on acupuncture treatment published from 2009 to 2015. We assessed the quality of reporting based on CAse REport (CARE) and STandards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) guideline checklists. *Results*. A total of 93 eligible case reports of acupuncture treatment were identified among the 107 articles screened. Overall quality of reporting in the case reports was generally acceptable (75.4% on CARE, 67.7% on STRICTA), but several crucial items remained substantially underreported. *Conclusions*. Endorsement of the CARE and STRICTA guidelines is needed to improve the completeness of reporting. Our findings will be helpful in developing a more appropriate reporting guideline for case reports in acupuncture treatment.

1. Introduction

Case reports are detailed narratives that describe a medical problem experienced by one or more patients for the purpose of medicine, science, or education [1]. They are considered useful for recognizing new diseases and identifying adverse events and beneficial effects associated with new treatments [2]. Since case reports are not sufficiently rigorous to show evidence of effectiveness in the era of clinical trials, they can be easily overlooked as "mere anecdotes" [3]. However, case reports not only guide personalized treatment in clinical practice, but they also generate hypotheses for future clinical trials [4]. In recent years, integrating systematically collected data from the real world by using sophisticated clinical research methods has been expected to uncover hidden evidence [4]. Thus, patient case reports can be valuable sources of new information that may lead to vital research and advances in clinical practice, in turn improving patient outcomes [5]. Given that acupuncture involves complex and varied forms of treatment, it is necessary to carefully record what happens in clinical practice [3].

The "CAse REport (CARE) guidelines" were proposed to facilitate systematic reporting of information in case reports [1]. It is widely expected that implementation of the CARE guidelines will improve the completeness and transparency of case reporting [1]. Guidelines for reporting adverse events of acupuncture were proposed in 2004, but there was no specific guideline for case reports about acupuncture treatment [6]. Recently, the Korean version of the CARE guidelines checklist was implemented in case reporting of acupuncture treatment [7]. The STandards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA), originally developed to improve the completeness and transparency of the reporting of interventions in controlled trials of acupuncture, are now expected to expand to encompass a broad range of clinical evaluation designs, including case reports [8, 9]. Since STRICTA recommendations comprise a checklist that expanded the generic content of Item 4 (reporting of intervention) of the CONSORT statement for controlled trials, it would also be necessary to follow the STRICTA guidelines for reporting acupuncture interventions in case reports. Author guidelines in journals suggest that full details

of the acupuncture treatment in case reports should follow the STRICTA criteria [3]. To the best of our knowledge, no study has investigated the reporting quality of case reports in acupuncture research in Korea based on the CARE and STRICTA guidelines.

Hence, we aimed to assess the current status of the reporting quality of case reports concerning acupuncture treatment in Korea based on the CARE and STRICTA guideline checklists.

2. Methods

2.1. Searching for and Selecting Case Reports. To assess the quality of reporting in acupuncture treatment case reports in Korea, all such reports were searched for in the Korean academic journal, *The Acupuncture (The Journal of Korean Acupuncture and Moxibustion Medicine Society)*. This journal is considered a core journal, which is highly cited in Korea [10]. Since this journal publishes acupuncture treatment case reports with separate subheadings, all case reports were retrieved based on the subheadings from January 2009 to September 2015. All case reports with acupuncture treatment as the intervention, regardless of the patient's diagnosis, were included in the analysis.

2.2. Data Extraction. Data were extracted independently by two assessors (Jeongjoo Kim and Yoon-Ji Eom) in accordance with prepared data extraction forms. As the STRICTA guidelines were originally developed to report the components of needling acupuncture, acupuncture was defined in this study as needle penetration of body points using manual and electrical stimulation [11]. To assess the quality of reporting of treatment components of acupuncture interventions in case reports, we used the revised version of the STRICTA guidelines published in 2010 [8]. Only the acupuncture-related information was extracted for analysis of the STRICTA items. The CARE guideline and STRICTA guidelines were converted into 31 and 15 checklist items, respectively, for data assessment. Before the evaluation, two assessors underwent training on each CARE and STRICTA item to ensure consistency in interpretation and scoring.

2.3. Evaluation of the CARE and SRTICTA Guideline Checklists. Items were worded closely to correspond to the original recommendations and rephrased as a series of questions. Each item from CARE and STRICTA was assessed as "yes" if it was included in the article or "no" if it was not. When at least one subitem was completely reported, the reporting item was counted as "yes." The interrater reliability was calculated using Cohen's kappa statistic for all items combined (kappa = 0.834 for CARE items; kappa = 0.729 for STRICTA items), and disagreements were resolved by joint discussion with a third assessor (Younbyoung Chae).

The CARE and STRICTA index was calculated to summarize the overall completeness of reporting by summing the scores for the 31 items of the CARE checklist and 15 items of the STRICTA guidelines [11, 12].

3. Results

3.1. Included Case Reports. In total, 93 of 107 screened case reports of acupuncture treatment were included for the assessment of reporting quality. Studies that combined acupuncture with other interventions were included, but those assessing only other interventions were excluded (n = 14) (Figure 1).

3.2. Quality of Reporting with CARE Guideline Items. The overall quality of reporting was relatively high (mean = 75.4%, 95% CI: 74.4 to 76.4) (Table 1). The CARE index was 23.4 (95% CI: 23.1 to 23.7). Items with markedly incomplete reporting (less than 50%) were diagnostic challenges (number 16, 2.2%), diagnostic reasoning including consideration of other diagnoses (number 17, 12.9%), prognostic characteristics (number 18, 25.8%), changes in intervention with rationale provided (number 21, 25.8%), intervention adherence and tolerability (number 24, 0%), adverse and unanticipated events (number 25, 16.1%), patient perspective (number 30, 29.0%), and informed consent (number 31, 12.9%).

3.3. Quality of Reporting with STRICTA Guideline Items. The quality of reporting of acupuncture interventions in case reports was evaluated according to the STRICTA guidelines. The overall quality of reporting was acceptable (mean = 67.7%, 95% CI: 64.8 to 70.6) (Table 2). The STRICTA index was 10.2 (95% CI: 9.7 to 10.6). Items with markedly incomplete reporting (less than 50%) were acupuncture regimen variation (number 3, 33.3%), depth of insertion (number 6, 46.2%), response sought (number 7, 23.7%), setting and context (number 14, 18.3%), and description of acupuncturists (number 15, 23.7%).

4. Discussion

A total of 93 case reports of acupuncture treatment in Korea were appraised in detail based on CARE and STRICTA guidelines. This study systematically illustrates the current reporting quality of case reports of acupuncture treatment. Quality of reporting was generally acceptable, but some items require further improvement. Our findings reveal the current status of the quality of reporting in case reports of acupuncture treatment in Korea and provide information that may facilitate the transparency and completeness of the reporting of case reports. Information obtained from transparent and detailed case reports would help provide a stronger basis for elucidation of the scope and effectiveness of acupuncture treatment, which in turn would be helpful in expanding the field of acupuncture research, as well as in developing further guidelines regarding clinical acupuncture practice.

With the prominent increase of case reports in medical journals, CARE guidelines provide a framework for a systematic reporting standard [4]. In the current study, the overall completeness of reporting of case reports in Korea was relatively high (75.4%), but several items were still lacking in the majority of the acupuncture treatment case reports. The reporting of diagnostic assessment items, such

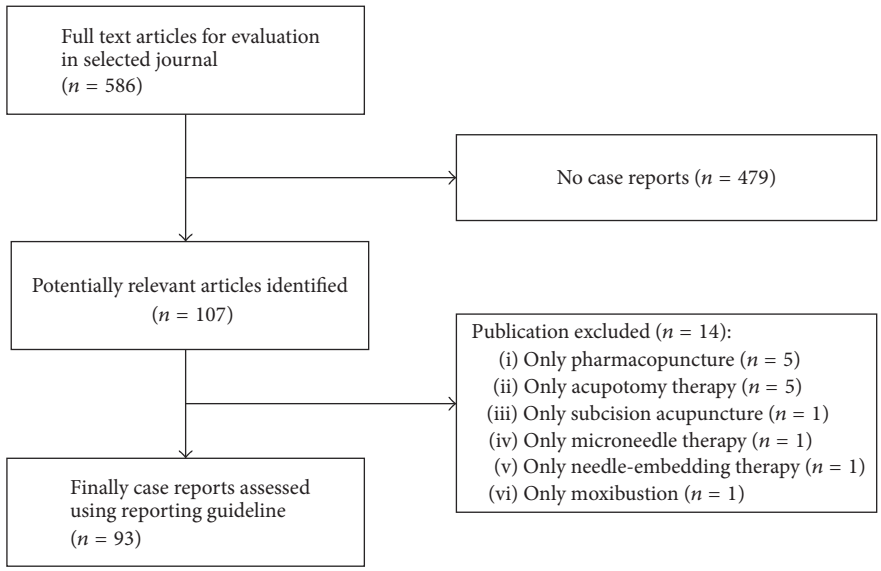

Figure 1: Flow chart of the articles identified, included, and excluded.

as diagnostic challenges (number 16), diagnostic reasoning including consideration of other diagnoses (number 17), and prognostic characteristics (number 8), was remarkably incomplete. Low quality reporting, particularly in the diagnostic assessment items, might be due to the dual medical system in Korea, in which Korean medical doctors are very limited in terms of their ability to use medical examination equipment. Concerning therapeutic intervention, changes in intervention (number 21) were reported in about 25.8% of the included case reports. Among the follow-up and outcome criteria, none of the acupuncture treatment case reports reported intervention adherence, and tolerability and adverse and unanticipated events were reported in only 16.1% of the included case reports. Items related to patient perspective (number 30) and informed consent (number 31) were markedly underreported. Collectively, these items should be more carefully presented in case reports.

In the present study, the quality of the reporting of intervention details based on STRICTA items was generally acceptable (67.7%) but still less complete than that based on CARE items. Among the poorly reported items (under 50%), acupuncture regimen variation (number 3) was reported by about one-third of the case reports. Insertion depth (number 6) and *de qi* response (number 7) were reported relatively infrequently, in 46.2% and 23.7% of case reports, respectively. Insertion depth and *de qi* response are important as the main specific components of acupuncture treatment [13–15]. To explore the causal relationship between acupuncture and outcome, these components should be much improved in acupuncture treatment case reports. Setting and context (number 14) and description of the acupuncturists (number 15) were considerably underreported, at 18.3% and 23.7%, respectively. These two variables are known to be nonspecific components of acupuncture treatment, and these items are considered less important in clinical trials [16–18]. One potential way to minimize the possible involvement of nonspecific effects in acupuncture treatment is through complete reporting of these items. Based on the STRICTA index in Korea, the reporting quality of case reports in the current study was similar to that of clinical trials in other studies [10, 11]. However, only 4 out of 93 case reports adopted STRICTA guidelines and reported all items. We strongly suggest that intervention details of acupuncture treatment in case reports be described according to STRICTA criteria.

Reporting guidelines play an important role in improving the quality of papers in clinical trials [11, 19–21]. The CARE guidelines were developed and translated into Korean to improve the completeness and transparency of case reports; however, they are not limited specifically to case reports of acupuncture treatment [1]. Recently, case reports involving traditional Chinese medicine (CARC) were developed based on a review of the general reporting quality of those reports, and through internal discussion by experts [22]. The CARC recommendations covered all traditional Chinese medicine interventions including Chinese herbal interventions, acupuncture, and moxibustion. Moreover, these recommendations suggest that items should include diagnosis by traditional Chinese medicine-based methods, according to symptoms, signs, and the characteristics of the tongue and pulse. According to the survey, 67.4% of case reports included the traditional Chinese medicine terms for diseases, and 88.9% reported syndrome differentiation [23]. These efforts in the CARC recommendations might reflect the perspective that acupuncture treatment is determined not just by diagnosis based on Western medicine, but also by pattern identification based on traditional Chinese medicine.

The reporting quality of case reports based on CARE was relatively good in the Korean literature, but the pattern identification process for determining the acupuncture treatment procedure was still poorly reported. The lack of reports, however, does not reflect the current process of acupuncture treatment, in which pattern identification is not in any way

Table 1: Percentage of case reports with complete reporting of CARE items.

		Item	n/N	(%)
Number 1	Title	The words "case report"	93/93	100.0
Number 2	Keywords	Key elements	93/93	100.0
Number 3	Abstract	Introduction: what does this add?	93/93	100.0
Number 4		Case presentation: main symptoms	47/93	50.5
Number 5		Case presentation: main clinical findings	91/93	97.8
Number 6		Case presentation: main diagnoses and interventions	93/93	100.0
Number 7		Case presentation: main outcomes	93/93	100.0
Number 8		Conclusion: main "take-away" lessons	93/93	100.0
Number 9	Introduction	Brief background summary of this case	93/93	100.0
Number 10	Patient information	Demographic information	93/93	100.0
Number 11		Main symptoms of the patient	93/93	100.0
Number 12		Medical, family, and psychosocial history	92/93	98.9
Number 13	Clinical findings	Physical examination findings	93/93	100.0
Number 14	Timeline	Depicts important dates and times	77/93	82.8
Number 15	Diagnostic assessment	Diagnostic methods	83/93	89.2
Number 16		*Diagnostic challenges*	*2/93*	*2.2**
Number 17		*Diagnostic reasoning including other diagnoses considered*	*12/93*	*12.9**
Number 18		*Prognostic characteristics*	*24/93*	*25.8**
Number 19	Therapeutic intervention	Type of intervention (e.g., pharmacologic, surgical, preventive)	93/93	100.0
Number 20		Administration of intervention (e.g., dosage, strength, duration)	93/93	100.0
Number 21		*Changes in intervention (with rationale)*	*24/93*	*25.8**
Number 22	Follow-up and outcomes	Clinician and patient-assessed outcomes	93/93	100.0
Number 23		Important follow-up test results	93/93	100.0
Number 24		*Intervention adherence and tolerability*	*0/93*	*0**
Number 25		*Adverse and unanticipated events*	*15/93*	*16.1**
Number 26	Discussion	Strengths and limitations of the management of this case	93/93	100.0
Number 27		The relevant medical literature	93/93	100.0
Number 28		The rationale for conclusions (assessments of cause and effect)	93/93	100.0
Number 29		The main "take-away" message	93/93	100.0
Number 30	*Patient perspective*	*Patient perspective or experience*	*27/93*	*29.0**
Number 31	*Informed consent*	*Informed consent*	*12/93*	*12.9**
		Average	70.3/93	75.4
		CARE index: mean (95% CI)	75.4 (74.4 to 76.4)	

Values are the number of case reports that included the item divided by the total number of eligible case reports; *less than 50%.

undervalued. Pattern identification is accepted as one of the key components for deciding the acupuncture treatment approach. For instance, in Saam acupuncture in Korea, pattern identification enables the selection of acupoints that are not only proximal, but also distal, to the symptom-related organs or body parts [24, 25]. Considering that the process of determining the method of acupuncture treatment is based on pattern identification in clinical practice, it is necessary to include additional, crucial information about the characteristics of the tongue and pulse, as well as pattern identification based on symptoms and signs. New guidelines specifically tailored toward case reports of acupuncture treatment and reflecting the whole process of clinical practice will be required in the future.

This study had several limitations. First, the results might not fully represent all Korean journals, as the case reports that were included were extracted from a single journal. Because this journal alone has adopted reporting guidelines, the quality of reports of randomized controlled trials in traditional medicine journals in Korea was assessed in a separate, previous study [10]. Considering the representativeness of this journal, of the field of acupuncture research in Korea, it is reasonable to assess the quality of reporting of case reports according to the papers published therein. Second, we did not compare the compliance rate for the quality of reporting following the publication of the CARE and STRICTA recommendations in Korea over time, because all case reports in this study were published after 2009. However, our findings could provide valuable information about the current, overall reporting quality for acupuncture case reports in Korea. Furthermore, a future study will be needed to compare changes in the reporting quality of case reports after endorsement of the CARE and STRICTA guidelines.

Table 2: Percentage of case reports with complete reporting of STRICTA items.

	Item	n/N	(%)
Number 1	Style of acupuncture	93/93	100.0
Number 2	Reasoning for treatments	69/93	74.2
Number 3	*Acupuncture regimen variation*	*31/93*	*33.3**
Number 4	Number of needles	76/93	81.7
Number 5	Name of points	90/93	96.8
Number 6	*Depth of insertion*	*43/93*	*46.2**
Number 7	*Response sought (e.g., de qi)*	*22/93*	*23.7**
Number 8	Needle stimulation (e.g., manual, electrical)	65/93	69.9
Number 9	Needle retention time	79/93	84.9
Number 10	Needle type (diameter, length, etc.)	88/93	94.6
Number 11	Number of treatment sessions	80/93	86.0
Number 12	Frequency and duration of treatment sessions	84/93	90.3
Number 13	Details of other interventions	85/93	91.4
Number 14	*Setting and context*	*17/93*	*18.3**
Number 15	*Description of acupuncturists*	*22/93*	*23.7**
	Average	62.9/93	67.7
	STRICTA index: mean (95% CI)	10.2 (9.7 to 10.6)	

Values are the number of case reports that included the item divided by the total number of eligible case reports; *less than 50%.

Classical medical texts, such as *Shanghanlun* and *Linzhengzhinanyian*, are enclosed with several case reports in East Asian medicine [22]. They include a delicate reporting form to record the diagnosis, principles of treatment, therapeutic outcome, and prognosis of practical cases. Owing to low reporting quality, however, the ability to study and analyze the underlying principles of East Asian medicine based on these case reports remains limited. Case reports are inherently unable to exclude the possibility that outcomes are due to natural factors or the effects of another intervention [3]. Without a relevant control group, it is difficult to ascertain the extent to which a given outcome was due specifically to the effects of acupuncture treatment and how much was attributable to nonspecific effects. However, most patients have symptoms that do not accord exactly with the diagnostic criteria strictly defined by researchers [26]. From the perspective of patient-centered medicine, it is emphasized that the patient is more than the sum of his or her diseases. As Hippocrates stated "I would rather know the person who has the disease than the disease the person has" [27]. Case reports are most valuable in the context of patient-centered medicine, since they describe the personal experiences of a specific practitioner and disseminate valuable clinical information about patients in a more vivid manner.

In sum, the overall reporting quality of case reports was generally acceptable, but several crucial items remained substantially underreported in Korean acupuncture treatment case reports. Endorsement of the CARE and STRICTA guidelines is needed to improve the completeness of reporting of acupuncture treatment-based case reports. Case reports with more transparency in their content, as well as sufficiently detailed information, would be more useful not only for the care of individual patients, but also for healthcare providers and the broader medical community. Our findings will be helpful in developing more appropriate reporting guidelines for case reports of acupuncture treatment.

Disclosure

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest

The authors have declared that no conflicts of interest exist.

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References

[1] J. J. Gagnier, G. Kienle, D. G. Altman et al., "The CARE guidelines: consensus-based clinical case reporting guideline development," *BMJ Case Reports*, 2013.

[2] M. Hauben and J. K. Aronson, "Gold standards in pharmacovigilance: the use of definitive anecdotal reports of adverse drug reactions as pure gold and high-grade ore," *Drug Safety*, vol. 30, no. 8, pp. 645–655, 2007.

[3] A. White, "Writing case reports—author guidelines for acupuncture in medicine," *Acupuncture in Medicine*, vol. 22, no. 2, pp. 83–86, 2004.

[4] D. Riley, "Case reports in the Era of clinical trials," *Global Advances in Health and Medicine*, vol. 2, no. 2, pp. 10-11, 2013.

[5] H. Cohen, "How to write a patient case report," *American Journal of Health-System Pharmacy*, vol. 63, no. 19, pp. 1888–1892, 2006.

[6] E. Peuker and T. Filler, "Guidelines for case reports of adverse events related to acupuncture," *Acupuncture in Medicine*, vol. 22, no. 1, pp. 29–33, 2004.

[7] S. M. Lee, Y. S. Shin, D. W. Nam, and D. Y. Choi, "Korean translation of the CARE guidelines," *The Acupuncture*, vol. 32, no. 4, pp. 1–9, 2015.

[8] H. MacPherson, D. G. Altman, R. Hammerschlag et al., "Revised STandards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA): extending the CONSORT statement," *PLOS Medicine*, vol. 7, no. 6, Article ID e1000261, 2010.

[9] H. MacPherson, A. White, M. Cummings, K. Jobst, K. Rose, and R. Niemtzow, "Standards for reporting interventions in controlled trials of acupuncture: the STRICTA recommendations," *Complementary Therapies in Medicine*, vol. 9, no. 4, pp. 246–249, 2001.

[10] J. Choi, J. H. Jun, B. K. Kang, K. H. Kim, and M. S. Lee, "Endorsement for improving the quality of reports on randomized controlled trials of traditional medicine journals in Korea: a systematic review," *Trials*, vol. 15, article 429, 2014.

[11] K. H. Kim, J. W. Kang, M. S. Lee, and J.-D. Lee, "Assessment of the quality of reporting in randomised controlled trials of acupuncture in the Korean literature using the CONSORT statement and STRICTA guidelines," *BMJ Open*, vol. 4, no. 7, Article ID e005068, 2014.

[12] C. Han, K.-P. Kwak, D. M. Marks et al., "The impact of the CONSORT statement on reporting of randomized clinical trials in psychiatry," *Contemporary Clinical Trials*, vol. 30, no. 2, pp. 116–122, 2009.

[13] Y. Chae and H. Olausson, "The role of touch in acupuncture treatment," *Acupuncture in Medicine*, vol. 35, no. 2, 2017.

[14] W. M. Jung, W. Shim, T. Lee et al., "More than DeQi: spatial patterns of acupuncture-induced bodily sensations," *Frontiers in Neuroscience*, vol. 10, article 462, 2016.

[15] H. M. Langevin, P. M. Wayne, H. MacPherson et al., "Paradoxes in acupuncture research: strategies for moving forward," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 180805, 11 pages, 2011.

[16] H. Chung, H. Lee, D.-S. Chang et al., "Doctor's attire influences perceived empathy in the patient-doctor relationship," *Patient Education and Counseling*, vol. 89, no. 3, pp. 387–391, 2012.

[17] T. J. Kaptchuk, J. M. Kelley, L. A. Conboy et al., "Components of placebo effect: randomised controlled trial in patients with irritable bowel syndrome," *British Medical Journal*, vol. 336, no. 7651, pp. 999–1003, 2008.

[18] I.-S. Lee, C. Wallraven, J. Kong et al., "When pain is not only pain: inserting needles into the body evokes distinct reward-related brain responses in the context of a treatment," *Physiology and Behavior*, vol. 140, pp. 148–155, 2015.

[19] L.-M. Lu, J. He, J.-C. Zeng, M.-X. Liao, C. Jia, and H.-H. Pan, "Impact evaluation of CONSORT and STRICTA guidelines on reporting quality for randomized controlled trials of acupuncture conducted in China," *Chinese Journal of Integrative Medicine*, pp. 1–8, 2016.

[20] B. Ma, Z. Chen, J. Xu et al., "Do the CONSORT and STRICTA checklists improve the reporting quality of acupuncture and moxibustion randomized controlled trials published in chinese journals? a systematic review and analysis of trends," *PLOS ONE*, vol. 11, no. 1, Article ID e0147244, 2016.

[21] S. L. Prady, S. J. Richmond, V. M. Morton, and H. MacPherson, "A systematic evaluation of the impact of STRICTA and CONSORT recommendations on quality of reporting for acupuncture trials," *PLoS ONE*, vol. 3, no. 2, Article ID e1577, 2008.

[22] S.-F. Fu, C.-W. Cheng, L. Zhang et al., "Consensus-based recommendations for case report in Chinese medicine (CARC)," *Chinese Journal of Integrative Medicine*, vol. 22, no. 1, pp. 73–79, 2016.

[23] S.-F. Fu, W. Kun, X.-X. Zeng et al., "Urgent need to improve the quality of case report in traditional chinese medicine: assessment on reporting quality of 3,417 cases," *Chinese Journal of Integrative Medicine*, vol. 22, no. 6, pp. 473–480, 2016.

[24] T. Lee, W. Jung, I. Lee et al., "Data mining of acupoint characteristics from the classical medical text: DongUiBoGam of korean medicine," *Evidence-Based Complementary and Alternative Medicine*, vol. 2014, Article ID 329563, 10 pages, 2014.

[25] M. Park and S. Kim, "A modern clinical approach of the traditional Korean Saam acupuncture," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 703439, 8 pages, 2015.

[26] P. P. Glasziou and L. M. Irwig, "An evidence based approach to individualising treatment," *The BMJ*, vol. 311, no. 7016, article 1356, 1995.

[27] J. Bensing, "Bridging the gap: the separate worlds of evidence-based medicine and patient-centered medicine," *Patient Education and Counseling*, vol. 39, no. 1, pp. 17–25, 2000.

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Research Article

Comparative Analysis of Tongue Indices between Patients with and without a Self-Reported Yin Deficiency: A Cross-Sectional Study

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We investigated the hypothesis that Yin-deficient patients have a reddened tongue with less coating. We screened 189 participants aged 20 to 49 years, complaining of headache. To classify patients in terms of Yin deficiency, we used two self-reporting Yin-deficiency questionnaires (Yin-Deficiency Questionnaire and Yin-Deficiency Scale) and diagnosis by a doctor. Based on the tests, a total of 33 subjects were assigned to a Yin-deficient group and 33 subjects were assigned to a nondeficient control group. Tongue images were acquired using a computerized tongue diagnostic system, for evaluating tongue indices. The tongue coating percentage and tongue redness were calculated as the mean a^* value of both the whole tongue area (WT a^*) and the tongue body area (TB a^*). The tongue coating percentage of the Yin-deficient group (34.79 ± 10.76) was lower than that of the nondeficient group (44.13 ± 14.08). The WT a^* value of the Yin-deficient group (19.39 ± 1.52) was significantly higher than that of the nondeficient group (18.21 ± 2.06). However, the difference in the TB a^* value between the two groups was not significant. In conclusion, we verified that Yin-deficient patients had less tongue coating and tended to have a more reddish tongue than nondeficient patients.

1. Introduction

In traditional Korean medicine (TKM) and traditional Chinese medicine (TCM), pattern identification is used to determine the pathological state of a patient. The patterns, called Zheng, imply the cause, nature, location, and the severity of the disease. Thus, a traditional medicine practitioner gathers and analyzes the symptoms and signs present in a patient to determine the course of action for treating an underlying disease. To identify such patterns, practitioners typically perform inspections that involve listening, smelling, inquiry, and palpation [1].

Inspection of the tongue is an important diagnostic approach in pattern identification in TKM because the tongue is considered to reflect the status of the human body, such as that of the qi-blood and the fluid of the internal organs, rapidly [2]. The practitioner examines the color, shape, moisture, and movement of the tongue body (which is a composite of muscles and vessels) and the color, thickness, and distribution of the tongue coating (a fur-like attachment to the dorsum of the tongue).

A Yin-deficiency (YD) pattern is a pathological state. The clinical symptoms of YD are typically associated with emaciation, tidal fever, night sweats, malar flush, palpitations, insomnia, dizziness, tinnitus, dry mouth and throat, constipation, a reddened tongue with little coating, and a fine rapid pulse [3]. In particular, a reddened tongue with little coating is regarded as a distinctive sign of YD. A "reddened tongue" sign indicates that the color of the tongue is reddish rather than the normal pale red color of the tongue, and a tongue with "little coating" indicates that either no or only a small amount of tongue coating is visible on the tongue body.

However, whether the sign of a reddened tongue with little coating is associated with YD in patients remains

unverified. Moreover, the definition of a "reddened tongue" has not been definitively established. A "reddened tongue" can be interpreted as the result of less coating on the tongue body, which reveals a pinker or redder color, or may reflect increased blood flow.

Several TKM and TCM studies [4–7] have investigated the tongue status of Yin-deficient patients. In those studies, the Yin-deficient patients were mainly grouped at the discretion of the practitioners; this methodology lacked objectivity. Thus, the results of these studies are not highly reliable. For objective assessment of a Yin-deficient status, several tools have been developed, such as the Yin-Deficiency Questionnaire (YDQ), the Yin-Deficiency Scale (YDS), Shi-pattern analysis, the Yin/Yang-deficiency Questionnaire, and the Yin-Xu Body Constitution Questionnaire [8]. Nevertheless, most of these tools do not include a tongue inspection or rely only on the subjective tongue inspection of the practitioner [9–11]. Some studies [12–15] have reported that inspection of the tongue has a low reliability, and thus an objective approach to inspection is needed. Therefore, several types of quantitative tongue assessment systems, such as a computerized tongue diagnostic system (CTDS), have been developed to solve this problem. Han et al. [16] investigated the relationship between tongue status and YD with the YDQ, using a CTDS; the results of this study were not consistent with the traditional medicine theory of tongue diagnosis. However, a single investigation is not sufficient to gain an understanding of the relationship between tongue status and YD.

Therefore, the present study quantitatively investigated the hypothesis that YD in patients is associated with a reddened tongue with little coating. We compared evaluation indices of the tongue between a Yin-deficient group of patients and a nondeficient control group. To identify patients with a YD and nondeficient patients, we used two self-reporting questionnaires, the YDS and YDQ [1, 17], both of which have been previously validated. To investigate differences in the tongue status between the two patient groups, we evaluated indices of the amount of tongue coating and the tongue color using a CTDS.

2. Materials and Methods

2.1. Ethics Approval and Consent to Participate. This study was conducted according to the standards of the International Committee on Harmonization of Good Clinical Practice and the revised version of the Declaration of Helsinki. Written informed consent was obtained from all of the study participants before the experiment. The Institutional Review Board of the Traditional Korean Medicine Hospital of Sangji University in Wonju, Republic of Korea, approved the experimental protocol (IRB number SJ IRB-Human-15-010).

2.2. Study Design. Between December 2015 and August 2016, a total of 189 people were screened from among the outpatients complaining of headache at the Traditional Korean Medicine Hospital of Sangji University in Wonju, Republic of Korea. All patients were aged between 20 and 49 years. The exclusion criteria were pregnancy, severe systemic organ diseases, use of drugs within the 7 days prior to screening, smoking, vitamin B use, or an abnormal condition of the tongue, that is, the inability to open the jaw and protrude the tongue stably, the presence of a geographic tongue, bleeding, malformation of the tongue, or the presence of dental braces. Thirty-one patients were excluded in the process of screening according to these criteria.

Demographic characteristics were recorded for each patient. In addition, the duration of the headache was recorded and severity of the headache was also assessed using a visual analog scale. After an experimenter explained the YDS and YDQ, each patient completed the questionnaires. To prevent any bias due to a misunderstanding of the questionnaires, a TKM doctor with more than 10 years of clinical experience diagnosed each patient as Yin-deficient or nondeficient, while blinded to the results of the questionnaires and without inspection of the patient's tongue. The diagnosis of a YD or a nondeficient status in patients was thus based on the scores of the YDS and the YDQ as well as the independent opinion of the doctor. Fifty-nine patients were excluded because of discordance between the two questionnaires. One YD subject was also excluded from our study based on the decision of the doctor that he was nondeficient. Then, the nondeficient patient group was matched to the group of patients who had YD, in terms of age and gender. Thirty-two nondeficient individuals were also excluded for matching. Finally, 66 participants were registered for this study. The overall design of the study is presented in Figure 1.

2.3. Tongue Examination. All subjects had their tongues examined using a CTDS. The subjects were required to avoid food and liquid intake for at least 4 h before the tongue examination and to refrain from brushing their teeth and tongue. In consideration of the influence of circadian rhythms, the tongue examination was conducted in the morning within 24 h of the screening. To image the tongue, the patients were instructed on how to touch their faces and protrude their tongues to the CTDS. After the patient exercised the procedure several times, opening their mouths and extruding the tongue to reveal their entire tongue, the CTDS was used to acquire an image of the tongue. To assess the reliability of the CTDS, an image of the tongue was acquired again in the same manner after 30 min. After image acquisition, we assessed whether the patients experienced any adverse event. The initial 66 images that were acquired during this examination were analyzed in a comparison of the YD patient group and the nondeficient patient group. The second group of images was only used to calculate the reliability of the CTDS.

2.4. The Yin-Deficiency Questionnaire and the Yin-Deficiency Scale. In 2007, the YDQ was developed for objective measurement of the severity of a YD [1]. The reliability and validity of the YDQ have been previously established [18], and the YDQ has been used to investigate the relationship between a YD and other symptoms, such as dry mouth, hot flush, and skin disorders [19–21]. The YDQ is comprised of 10 questions to which the patients responded by marking the

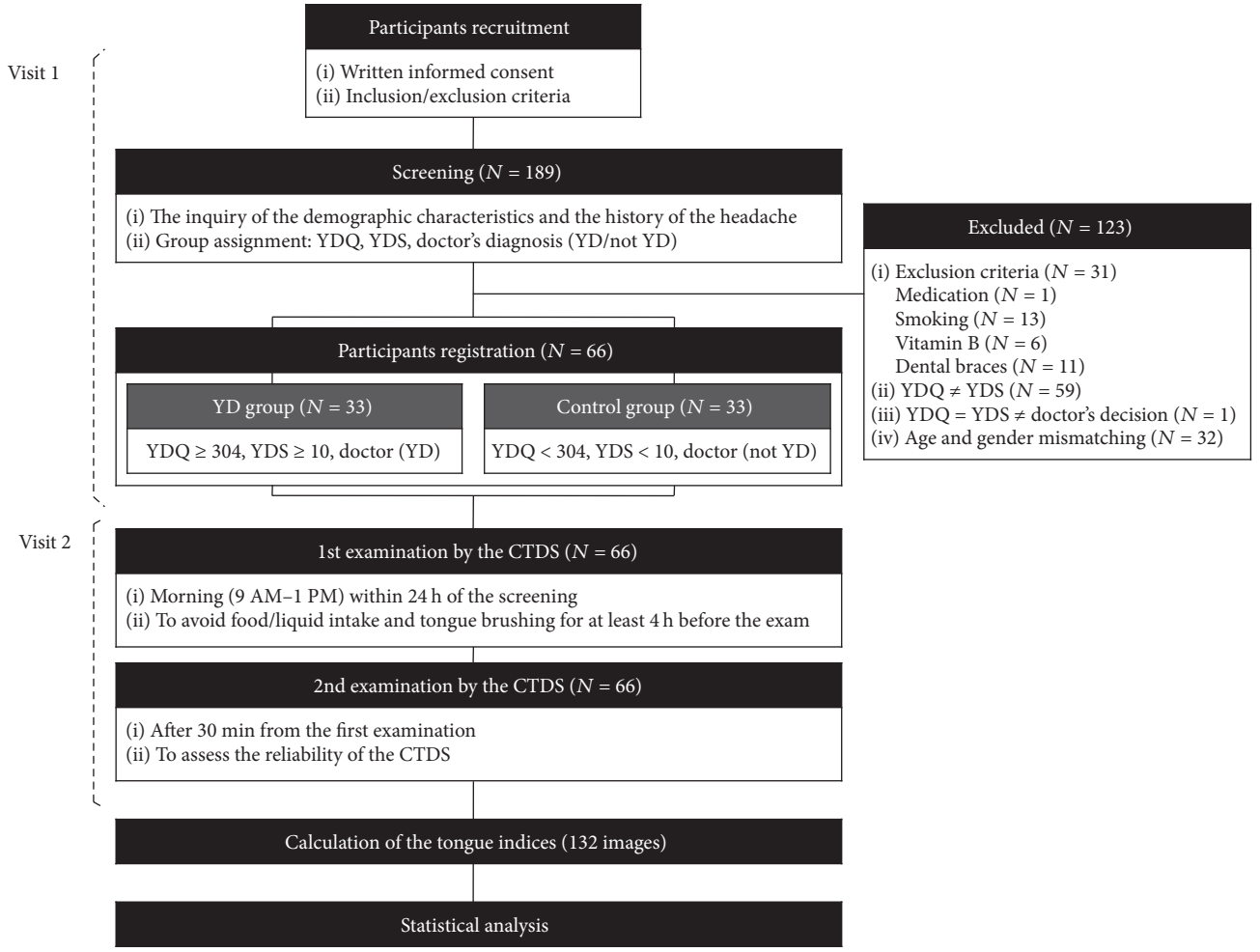


Figure 1: Flow chart of the experimental design of the present study. YDS: Yin-Deficiency Scale; YDQ: Yin-Deficiency Questionnaire; YD: Yin deficiency; CTDS: computerized tongue diagnostic system.

severity of their symptoms on a 100 mm bar. The cut-off score for a YD diagnosis is 304 [22].

Park et al. [17] developed the YDS, which suggests the optimum cut-off score for a diagnosis of YD as 10. The YDS includes a total of 27 questions to which the patients responded on a scale of 1 to 7, to indicate the severity of a YD. To discriminate patients with YD, the score of the YDS was calculated on a dichotomous scale; the scores of 1, 2, 3, and 4 were converted to 0 points and the scores of 5, 6, and 7 were converted to 1 point.

2.5. Image Acquisition System. A CTDS (CTS-1000, Daiseung Medics, Seoul, South Korea) was used to acquire tongue images. The CTDS comprised a camera, illuminators, an external light shielding system, and analysis software. The camera was set up using a color board (Color Checker Passport, X-Rite, Grand Rapids, MI, USA) before the capture of tongue images. The operator captured an image of the tongue together with two 18% gray chips (R27, Kodak, Rochester, NY, USA) on the bilateral sides of the tongue, to evaluate the gray balance and perform fast color adjustments. Additional details of the procedure for image acquisition using the CTDS are described in a previous study [23].

2.6. Calculation of the Tongue Indices. The region of the tongue was extracted from the captured image. The tongue coating area was distinguished from the tongue body area based on the difference between the a^* value for the tongue body and tongue coating. The RGB color values of each pixel in the areas were converted to CIE-$L^*a^*b^*$ color values. Then, the mean values of $L^*a^*b^*$ for the whole tongue (WT) area and tongue body (TB) area were calculated. The tongue coating percentage was calculated as the percentage of the pixel number of the tongue coating area to the pixel number of the whole tongue area. The process for calculating these tongue indices is shown in Figure 2. The tongue coating percentage was used as the index to estimate the amount of tongue coating. The redness of the tongue was estimated as the mean a^* value for the whole tongue area (WT a^*) and the mean a^* value for the tongue body area (TB a^*).

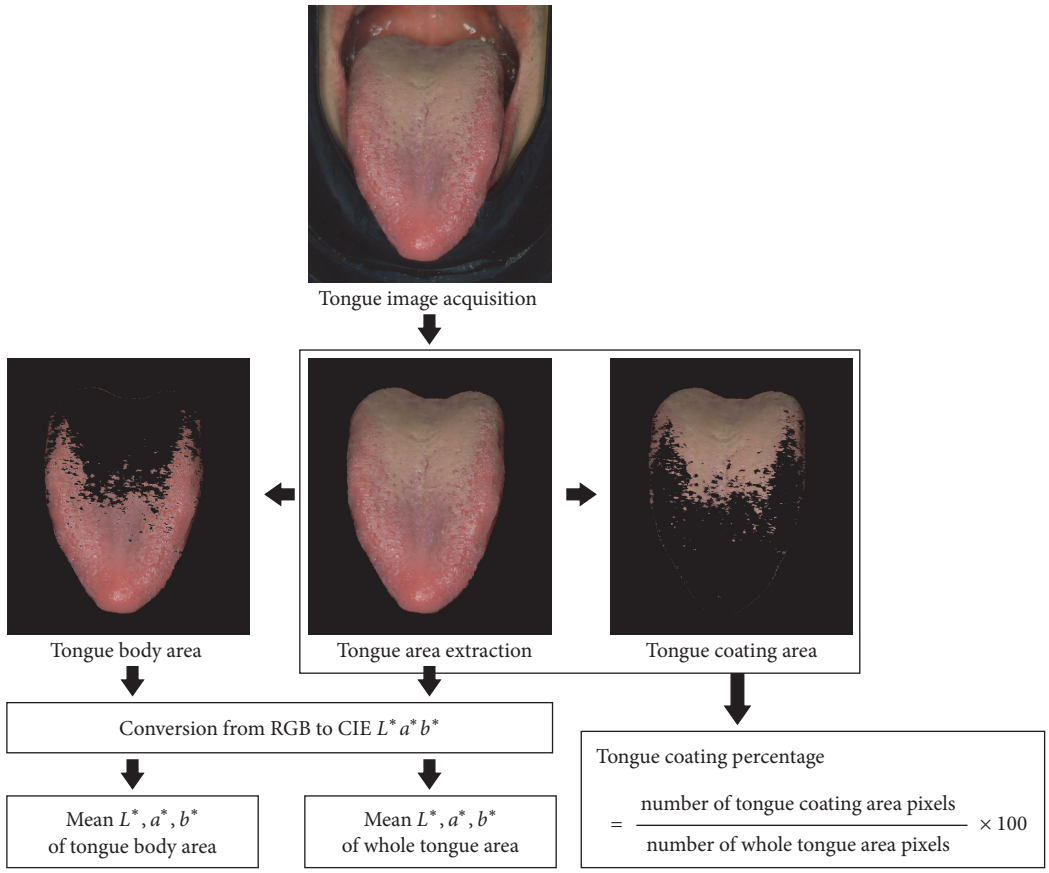


Figure 2: Calculation of tongue indices. The tongue area was extracted from an acquired tongue image. The tongue coating area was distinguished from the tongue body area based on the difference in color. The RGB color values in the areas of interest were converted into CIE-$L^*a^*b^*$ color values. Then, the mean $L^*a^*b^*$ values of whole tongue area and tongue body area were calculated. The tongue coating percentage was calculated as the percentage of the pixel number of the tongue coating area to the pixel numbers of the whole tongue area.

2.7. Statistical Analysis. The baseline characteristics of the participants are presented using descriptive statistics. Differences in the demographic characteristics and the severity of the headache between the YD group and nondeficient control group were compared using a chi-squared test for categorical data and an independent samples t-test for continuous data. If the assumption of normality was not confirmed, Mann–Whitney U test was used. To evaluate the homogeneity of the headache duration, we used the proportional odds model [24]. Levene's test was performed to assess the equality of variances for the demographic characteristics and the tongue indices between the two groups. Intraclass correlation coefficients (ICCs) were calculated to assess the reliability of the CTDS. ICC values above 0.8 indicate acceptable reliability according to Shrout and Fleiss [25]. A binary logistic regression analysis was performed to predict the probability of YD occurrence according to the indices. Statistical analyses were performed using IBM SPSS Statistics 23 (IBM Corporation, Armonk, NY, USA) or SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). A p value < 0.05 was considered statistically significant.

3. Results

3.1. Baseline Characteristics of the Participants. The baseline characteristics of the participants are shown in Table 1. The demographic differences between the YD group and nondeficient control group were not significant. In addition, differences in headache duration between the two groups were not significant; the estimated coefficient of the group effect was 0.484 (standard error = 0.45) and the corresponding p value was 0.280. However, the headache severity of the YD group was significantly higher than that of the nondeficient group.

3.2. Tongue Indices. The results for all of the tongue indices between the YD group and nondeficient control group are presented in Table 2. The tongue coating percentage of the YD group (34.79 ± 10.76) was significantly lower than that of the nondeficient group (44.13 ± 14.08; $p = 0.004$). The WT a^* value was significantly different between the two groups ($p = 0.010$); the WT a^* value of the YD group (19.39 ± 1.52) was higher than that of the nondeficient group (18.21 ± 2.06). However, there was no significant difference in the TB a^*

Table 1: Baseline characteristics of the participants.

	Yin deficiency ($N = 33$)	Control ($N = 33$)	Total ($N = 66$)	p value
Sex (M/F)[(a)]	10 (30.3)/23 (69.7)	12 (36.4)/21 (63.6)	22 (33.3)/44 (66.7)	0.602
Age (years)[(b)]	30.82 ± 7.49	30.82 ± 8.71	30.82 ± 8.06	1.000
Height (cm)[(c)]	165.99 ± 6.31	165.97 ± 9.27	165.98 ± 7.87	0.993
Weight (kg)[(b)]	63.67 ± 12.85	63.26 ± 12.52	63.46 ± 12.59	0.896
Systolic blood pressure (mmHg)[(b)]	107.61 ± 8.51	111.42 ± 13.90	109.52 ± 11.59	0.183
Diastolic blood pressure (mmHg)[(b)]	72.36 ± 6.67	74.30 ± 10.82	73.33 ± 8.97	0.385
Pulse rate (bpm)[(b)]	68.36 ± 10.98	66.58 ± 8.75	67.47 ± 9.89	0.467
Temperature (°C)[(b)]	36.46 ± 0.19	36.40 ± 0.18	36.43 ± 0.18	0.140
Headache duration				
Duration < 1 week	6 (18.2)	12 (36.4)		
1 week ≤ duration < 1 month	10 (30.3)	5 (15.2)	0.48 ± 0.45	0.280
1 month ≤ duration < 6 months	4 (12.1)	6 (18.2)		
6 months ≤ duration	13 (39.4)	10 (30.3)		
Headache VAS[(b)]	4.55 ± 1.86	3.42 ± 1.95	3.98 ± 1.97	0.020*

Data are presented as n (%) or mean ± SD and were compared by chi-squared test[(a)], independent samples t-test[(b)], or Mann–Whitney U test[(c)]. Headache duration was compared by estimated coefficient of group effect and mean ± SE; $^{*}p < 0.05$.

Table 2: Tongue indices.

Variables	Levene's test		Yin deficiency ($N = 33$)	Control ($N = 33$)	p value
	F	p value			
Tongue coating percentage (%)	3.195	0.079	34.79 ± 10.76	44.13 ± 14.08	0.004**
Tongue body area					
L^*	1.352	0.249	51.72 ± 2.41	50.50 ± 2.83	0.064
a^*	0.892	0.349	22.38 ± 1.17	22.26 ± 1.61	0.729
b^*	0.209	0.649	10.77 ± 1.81	11.39 ± 2.02	0.194
Whole tongue area					
L^*	0.893	0.348	50.07 ± 2.23	49.51 ±2.79	0.373
a^*	1.937	0.169	19.39 ± 1.52	18.21 ± 2.06	0.010**
b^*	0.107	0.745	10.30 ± 1.73	10.84 ± 2.00	0.244

The variables were compared by an independent samples t-test; $^{**}p < 0.01$.

values between the two groups ($p = 0.729$). The differences in the tongue indices are presented in Table 2 and Figure 3.

3.3. The Reliability of the Computerized Tongue Diagnostic System. Independent examination of two images per participant revealed the acceptable reliability of the CTDS, as shown in Table 3. All the ICCs of the tongue coating percentage and the $L^*a^*b^*$ values of the tongue body and whole body exceeded 0.8 (tongue coating percentage = 0.826; the TB a^* value = 0.801; the WT a^* value = 0.812).

3.4. Binary Logistic Regression. We conducted binary logistic regression for YD with the tongue coating percentage and the WT a^* value. Table 4 and Figure 4 show the regression model for the tongue coating percentage. The Wald test indicated that the tongue coating percentage was a significant predictor of YD ($p = 0.007$). In the regression model, the WT a^* value was removed because it was not significant as a predictor of YD ($p = 0.759$) once the tongue coating percentage was included in the model. The effective coating percentage for which there will be a 50% chance of YD is 39.47% (2.368/0.06).

3.5. Adverse Event. None of the patients experienced any adverse events during the study period.

4. Discussion

The results of the present study showed that the tongue coating percentage of the YD group was lower and that

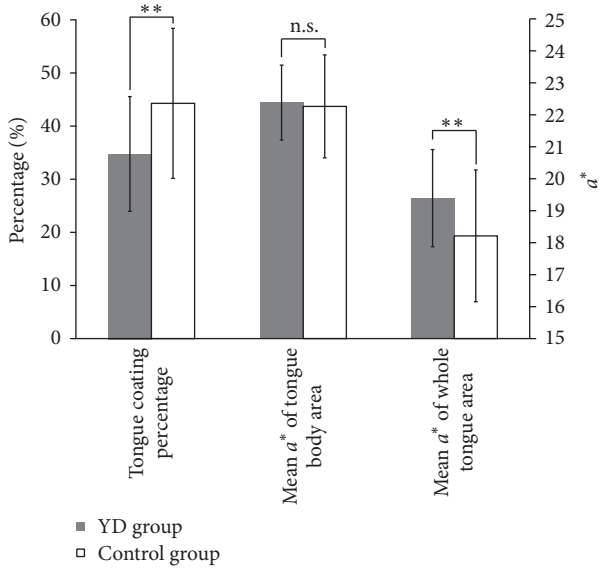


Figure 3: Comparison of the tongue indices between the Yin-deficient group and the nondeficient control group. The tongue coating percentage of Yin-deficiency (YD) group (34.79%) was significantly lower than that of the control group (44.13%). The mean a^* value of whole tongue area (WT a^*) was significantly different between the two groups; the WT a^* value of the Yin-deficient group (19.39 ± 1.52) was higher than that of the control group (18.21 ± 2.06). However, the mean a^* value of tongue body was not significantly different between the two groups. Vertical bars: ±SD; $^{**}p < 0.01$; n.s.: not significant.

Table 3: The reliability of the computerized tongue diagnostic system.

	ICCs	Standard error (95% CI)
Tongue coating percentage	0.826	0.715–0.893
Tongue body area		
L^*	0.800	0.673–0.877
a^*	0.801	0.675–0.878
b^*	0.865	0.779–0.917
Whole tongue area		
L^*	0.805	0.682–0.881
a^*	0.812	0.694–0.885
b^*	0.876	0.797–0.924

the WT a^* value was higher than those of the nondeficient control group. These results are consistent with the TKM tongue diagnosis theory that the tongue in patients with YD is reddened with little coating compared to the tongues of nondeficient individuals. However, we did not observe any significant difference in the TB a^* values between the YD and control groups. This negative result suggests that a reddish color of the tongue is the result of a decrease in the tongue coating on the body of the tongue. Representative tongue image examples from the YD patient and the nondeficient control are shown in Figure 5.

Previous studies have found negative results when attempting to verify TKM and TCM theories. However, these

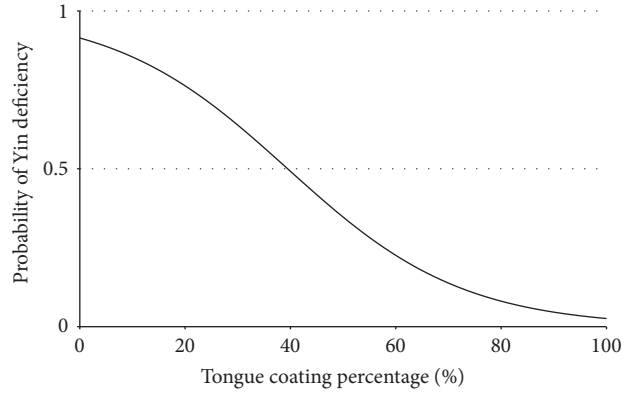


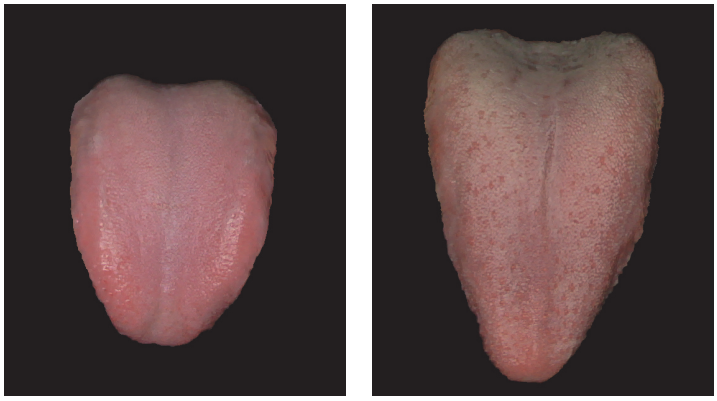
Figure 4: Logistic regression curve. Decrease of tongue coating percentage indicates an increased probability of Yin deficiency. [Probability of Yin deficiency = $\exp(0.06X - 2.368)/(1 + \exp(0.06X - 2.368))$; X = tongue coating percentage (%)].

negative results may have been due to the lack of objectivity in the methodology used in these studies; for most of these studies, the researchers subjectively evaluated the pattern and the tongue status, which likely biased their conclusions. In contrast, the present investigation used a more objective approach. We used two self-reporting questionnaires, in addition to the expert opinion of a practitioner to minimize the influence of experimenter bias, or the influence of an inaccurate self-report due to misunderstanding of the questionnaires. The patients that we identified as Yin-deficient complained of symptoms related to a YD; thus, our approach was likely objective and accurate.

Another strength of our study was the use of a CTDS to evaluate the tongue. With this approach, the conditions for obtaining tongue images remained constant. Moreover, our CTDS allowed a quantitative evaluation of tongue indices. The high ICC level demonstrates the reliability of our study.

In order to compare the tongue status with different patterns, the participants should have the same chief complaint but should be differentiated by variable patterns including YD. Therefore, we recruited patients who were all suffering from headache because the clinical pattern of a headache in TKM theory varies: wind-cold, wind-heat, wind-dampness, ascendant hyperactivity of the liver yang, static blood, heat syncope, dampness-phlegm, qi-deficiency, blood deficiency, and YD [26].

Previous studies have suggested that the amount of tongue coating increases with aging [27–30]; therefore, the participants in our study were limited to an age range of 20 to 49 years, and the control group was age-matched to the YD group. Consequently, the mean and standard deviation of age for both the YD group and the control group were similar. In addition, some studies have suggested that circadian rhythms influence oral status [31–33]; therefore, all of the examinations in our study were performed at approximately the same time in the morning. Moreover, our pilot study (not published) found that the correlation between the responses to the YD questionnaires and the tongue indices was weaker as time



Score of YDQ: 598
Score of dichotomous YDS: 20
Tongue coating percentage: 9.72%
Mean a^* of tongue body area: 25.2
Mean a^* of whole tongue area: 24.2

(a)

Score of YDQ: 35
Score of dichotomous YDS: 0
Tongue coating percentage: 43.82%
Mean a^* of tongue body area: 21.5
Mean a^* of whole tongue area: 17.4

(b)

Figure 5: Representative tongue images from the Yin-deficient and control groups. A tongue coating percentage was used as an index to estimate the amount of tongue coating. The redness of the tongue was measured as the mean a^* value of both the whole tongue area and the tongue body area that remained after excluding the tongue coating area. (a) A tongue image of a Yin-deficient patient is shown, and (b) a tongue image from a nondeficient control patient is shown. Note that these examples show that the tongue coating percentage of the Yin-deficient patient is lower and that the mean a^* value of the whole tongue area is higher than those of the nondeficient patient. The mean a^* value of the tongue body area showed a relatively small difference between the two samples.

Table 4: Binary logistic regression coefficients.

Model	B	SE	Wald	df	p value	exp(B)	95% CI for exp(B)	
							Lower	Upper
Tongue coating percentage	−0.060	0.022	7.354	1	0.007	0.941	0.901	0.983
Constant	2.368	0.903	6.885	1	0.009	10.678		

progressed after completing the questionnaires; therefore, we conducted our examination within 24 h of the screening.

We also performed Pearson's correlation coefficient analysis to investigate relationships among the tongue indices and the YDQ and YDS scores. We found that the tongue coating percentage and test scores for YD were negatively correlated. Moreover, the WT a^* values were positively correlated with the test scores for YD. However, this correlation was weak, as the correlation coefficients were between 0.25 and 0.35. This result might be due to the lack of severity in the group of YD patients; the mean total YDQ scores and the dichotomized YDS score for the YD patients were not high (YDQ = 485.5 ± 94.4; YDS = 14.4 ± 2.9), considering that the highest scores achievable on these tests are 1,000 and 27, respectively. The WT a^* values were very strongly correlated with the tongue coating percentage ($r = -0.922$, $p < 0.001$) and were not a significant predictor of YD when the tongue coating percentage was included in the logistic regression model. This implies that a "reddened tongue" can be interpreted as being the result of reduced coating on the tongue body, rather than the result of a reddened tongue body in the present study. Future studies should investigate patients with more severe YD to investigate whether a stronger correlation might appear.

The present study has several limitations. First, as mentioned above, the self-reporting screening could have been inaccurate due to the participant's misunderstanding of the questionnaire. Second, the number of participants that were included in our study was based on previous studies of tongue coating. Thus, our study design was more suitable for the investigation of tongue coating than of tongue color. Finally, although patterns of deficiency might be more easily observed in the elderly, the participants in the present study were relatively young, to increase the generalizability of the results.

5. Conclusions

Our results show that the tongue coating in self-reported YD patients tended to be decreased and the overall tongue color tended to be reddish compared to that in a nondeficient

control group. These observations are consistent with traditional medicine theory of tongue diagnosis.

Abbreviations

CTDS: Computerized tongue diagnostic system
TB: Tongue body area
TB a^*: The mean a^* value for the tongue body area
TCM: Traditional Chinese medicine
TKM: Traditional Korean medicine
WT: Whole tongue area
WT a^*: The mean a^* value for the whole tongue area
YD: Yin deficiency
YDS: Yin-Deficiency Scale
YDQ: Yin-Deficiency Questionnaire.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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References

[1] S. Lee, J. Park, H. Lee, and K. Kim, "Development and validation of Yin-Deficiency questionnaire," *American Journal of Chinese Medicine*, vol. 35, no. 1, pp. 11–20, 2007.

[2] B. Lee, Y. Park, and T. Kim, *Diagnosis in Traditional Korean Medicine*, Seongbosa, Seoul, Republic of Korea, 2000.

[3] WHO Regional Office for the Western Pacific, *Standard Terminologies on Traditional Medicine in the Western Pacific Region*, Renouf Publishing Co Ltd., Manila, Philippines, 2007.

[4] Z. L. Chen and Q. F. Hu, "Recent development in research on tongue inspection," *Chinese Medical Journal*, vol. 99, no. 6, pp. 444–456, 1986.

[5] S. Q. Zhang, "Tongue temperature of healthy persons and patients with Yin deficiency by using thermal video," *Chinese Journal of Modern Developments in Traditional Medicine*, vol. 10, no. 12, pp. 732-733, 1990.

[6] Q. F. Hu and Z. L. Chen, "Study on the mechanism of denuded tongue coating due to yin deficiency," *Chinese Journal of Modern Developments in Traditional Medicine*, vol. 9, no. 3, pp. 153–155, 1989.

[7] W. Su, Z. Y. Xu, Z. Q. Wang, and J. T. Xu, "Objectified study on tongue images of patients with lung cancer of different syndromes," *Chinese Journal of Integrative Medicine*, vol. 17, no. 4, pp. 272–276, 2011.

[8] S.-W. Park and D.-H. Nam, "A survey on utilization of questionnaires for assessing statues of Yin deficiency syndrome," *The Journal of the Society of Korean Medicine Diagnostics*, vol. 18, no. 2, pp. 63–74, 2014.

[9] S.-C. Lin, M.-F. Chen, T.-C. Li, Y.-H. Hsieh, and S.-J. Liu, "The distribution of yin-deficient symptoms and their relationship on survival rate in cancer patients with Yin-Deficiency," *American Journal of Chinese Medicine*, vol. 36, no. 4, pp. 655–663, 2008.

[10] S.-C. Lin, M.-L. Huang, S.-J. Liu, Y.-F. Huang, S.-C. Chiang, and M.-F. Chen, "Severity of Yin deficiency syndrome and autonomic nervous system function in cancer patients," *Journal of Alternative and Complementary Medicine*, vol. 15, no. 1, pp. 87–91, 2009.

[11] S.-C. Lin and M.-F. Chen, "Increased yin-deficient symptoms and aggravated autonomic nervous system function in patients with metastatic cancer," *Journal of Alternative and Complementary Medicine*, vol. 16, no. 10, pp. 1059–1063, 2010.

[12] M. Kim, D. Cobbin, and C. Zaslawski, "Traditional Chinese medicine tongue inspection: an examination of the inter- and intrapractitioner reliability for specific tongue characteristics," *Journal of Alternative and Complementary Medicine*, vol. 14, no. 5, pp. 527–536, 2008.

[13] M. M. Ko, J. A. Lee, B.-K. Kang, T.-Y. Park, and M. S. Lee, "Inter-observer reliability of tongue diagnosis using traditional Korean medicine for stroke patients," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 209345, 6 pages, 2012.

[14] J. Kim, G. Han, S.-J. Ko et al., "Tongue diagnosis system for quantitative assessment of tongue coating in patients with functional dyspepsia: a clinical trial," *Journal of Ethnopharmacology*, vol. 155, no. 1, pp. 709–713, 2014.

[15] I. Gareus, T. Rampp, L. Tan et al., "Introducing a computer-assisted, digital tongue-imaging device for standardization of traditional tongue diagnosis," *Forschende Komplementarmedizin*, vol. 21, no. 3, pp. 190–196, 2014.

[16] G. Han, J.-W. Park, S.-J. Ko et al., "Study on tongue coating patterns of the xerostomia in the elderly patients," *The Journal of the Society of Korean Medicine Diagnostics*, vol. 17, no. 3, pp. 189–202, 2013.

[17] Y.-J. Park, S.-W. Cho, B.-H. Lee, and Y.-B. Park, "Development and validation of the yin deficiency scale," *Journal of Alternative and Complementary Medicine*, vol. 19, no. 1, pp. 50–56, 2013.

[18] S. Lee, J. Park, and K. Kim, "Development of Yin-deficiency questionnaire and examine the reliability and validity," *Korean Journal of Oriental Physiology & Pathology*, vol. 18, no. 2, pp. 376–380, 2004.

[19] J. Kim, J. Kim, J. Park, and B. Ryu, "Availability of diagnosis of yin-deficiency in elderly people with xerostomia and factors influencing subjective oral dryness: a prospective cross-sectional study," *Journal of Korean Medicine*, vol. 34, no. 3, pp. 13–24, 2013.

[20] J. Kim, J. Jo, S. Yoo et al., "A study on relations between MRS, MENQOL, HRV, Yin-deficiency questionnaire in menopausal woman with hot flush," *The Journal of Oriental Obstetrics & Gynecology*, vol. 24, no. 4, pp. 71–84, 2011.

[21] G. Cho, S. Yu, E. Kim et al., "Change of Yin-deficiency scale and the skins physical properties based on female's aging pattern by 7 years," *The Journal of Korean Medical Institute of Dermatology & Aesthetics*, vol. 3, no. 1, pp. 24–35, 2010.

[22] S. Jang and J. Kim, "Development cut-off value for Yin-deficiency questionnaire and diagnositic ability of Yin-deficiency in xerostomia," *Journal of Internal Korean Medicine*, vol. 35, no. 4, pp. 483–497, 2014.

[23] J. Kim, J. Son, S. Jang et al., "Availability of tongue diagnosis system for assessing tongue coating thickness in patients with functional dyspepsia," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 348272, 6 pages, 2013.

[24] A. Agresti, *Categorical Data Analysis*, Wiley Series in Probability and Mathematical Statistics, John Wiley & Sons, New York, NY, USA, 2013.

[25] P. E. Shrout and J. L. Fleiss, "Intraclass correlations: uses in assessing rater reliability," *Psychological Bulletin*, vol. 86, no. 2, pp. 420–428, 1979.

[26] The Association of Department of Korean Heart Internal Medicine, *Cardiology and Neurology of Korean Traditional Internal Medicine*, Koonja Publishing Inc, Seoul, Republic of Korea, 4th edition, 2011.

[27] W. J. Ralph, "Hygiene of the tongue," *Gerodontics*, vol. 3, no. 4, pp. 169-170, 1987.

[28] W. J. Ralph, "Oral hygiene–why neglect the tongue?" *Australian Dental Journal*, vol. 33, no. 3, pp. 224-225, 1988.

[29] K. Yaegaki and K. Sanada, "Volatile sulfur compounds in mouth air from clinically healthy subjects and patients with periodontal disease," *Journal of Periodontal Research*, vol. 27, no. 4, pp. 233–238, 1992.

[30] M. Quirynen, C. Mongardini, and D. Van Steenberghe, "The effect of a 1-stage full-mouth disinfection on oral malodor and microbial colonization of the tongue in periodontitis patients. A Pilot Study," *Journal of Periodontology*, vol. 69, no. 3, pp. 374–382, 1998.

[31] H. Miyazaki, S. Sakao, Y. Katoh, and T. Takehara, "Correlation between volatile sulphur compounds and certain oral health measurements in the general population," *Journal of Periodontology*, vol. 66, no. 8, pp. 679–684, 1995.

[32] K. Ikawa, M. Iwakura, J. Washio, A. Kusano, N. Tanda, and T. Koseki, "Circadian changes of volatile sulfur compounds measured by Breathtron™," *International Congress Series*, vol. 1284, pp. 89-90, 2005.

[33] S. Supriatno, "The effect of circadian rhythm to Oral volatile sulfur compounds production," *Majalah Kedokteran Gigi Indonesia*, vol. 20, no. 1, pp. 14–20, 2013.

[34] S. Kim, *Comparative analysis of tongue indices between patients with and without a self-reported Yin-deficiency: a cross-sectional study [M.S. thesis]*, Sangji University, 2017.

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Research Article

Characteristics of Herbal Medicine Users and Adverse Events Experienced in South Korea: A Survey Study

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Background. This survey aimed to investigate the characteristics of users and nonusers of herbal medicine and the adverse events experienced due to herbal medicines in South Korea. *Methods.* The questionnaire consisted of safety, using experience, using type, usage and nonusage reason, purchase location, and adverse events of herbal medicine. The survey was administered by online. *Results.* Of the total 1,134 respondents, 726 (64.0%) considered herbal medicine safe, and 693 (61.1%) answered that they have taken herbal medicines within the past year. Most common place to purchase them was "TKM hospital or clinic" (63.6%), and most participants (72.2%) took a decoction from a TKM institution. The biggest reason for taking them was for "health improvement" (57.3%), and the reasons for not using them was "medication not necessary" (63.7%). Among those who took herbal medicines, 46 experienced adverse events, and the most frequently reported symptoms were digestive disorders (52.2%). Of the 46 participants who experienced adverse events, 20 (43.5%) were treated by TKM doctors. *Conclusions.* This study suggests that regulation of herbal medicines is needed in order to resolve problems related to the safety of herbal medicines.

1. Background

The global herbal medicine market has grown every year, and the total estimated retail sales of herbal supplements in the United States reached almost $7 billion after increasing by 7.5% in 2015 [1]. The sales are anticipated to further grow to reach $5 trillion by 2050 [2].

As the interest in herbal medicines increases, there is a growing need to ensure their safety. The goal of the World Health Organization (WHO) Traditional Medicine Strategy is to promote the safe and effective use of Traditional and Complementary Medicine (T&CM). Regulations on the safety of herbal medicine have increased in order to achieve this goal. The numbers of policies and regulations to ensure the safety and efficacy of T&CM were 69 and 119, respectively [3]. According to the Report on Usage and Consumption of Korean Medicines 2011 [4], approximately 22.4% of people who have experienced Korean Medicine hospitals or clinics answered that ensuring the safety of herbal medicines would lead to future improvements in Korean Medicine.

South Korea is a country with a long history of herbal medicine usage. However, until recently, there has not been a systematic report of adverse events, which would be the foundation of enhancing safety. According to the data of

Korean Regional Pharmacovigilance Centers in 2007, there was only one case of adverse drug reaction reported from herbs among the observed 1,418 cases [5]. As a result of self-investigation of a university hospital, 28 adverse drug reactions occurred from herbal medicines in one Korean Medicine Hospital in South Korea from January 1, 2008, to February 29, 2012 [6].

This study surveyed in detail the characteristics of consumers in Korea and the adverse events from herbal medicines, as well as the perceived safety of products. The objective of this study was to investigate Korean consumers' usage patterns with respect to herbal medicines and to provide research-based evidence for enhancing their safety.

2. Methods

2.1. Study Design and Setting. This study was a survey of the characteristics of herbal medicine users in South Korea. The survey was conducted by Macromill Embrain (http://www.embrain.com), which is a professional survey research company that manages about 1,180,000 online research panels in South Korea. The company recruited the participants with consideration of the age and sex distributions and informed them that responses had to be filled out for all our questions in the questionnaire. The participants were enrolled on a voluntary basis and there was no refusal rate. The survey was conducted anonymously between October 1 and 31, 2015.

2.2. Participants. There was no special method for determining the sample size; we only sought to have as many people as possible complete the survey during the survey period. The minimum number of participants was determined to be 1,000, with additional recruitment on-going until the end of the study period. The participants were stratified based on gender and age to get the status of general population. Those under 20 years old and over 70 years old were excluded.

2.3. Questionnaire. The questionnaire was developed by five traditional Korean medicine (TKM) experts who discussed and selected investigation items. A draft questionnaire was developed through two rounds of review, placing emphasis on the easy comprehension of the questionnaire to gear to the general population. The experts examined face reliability as well as readability of the questionnaire. Then, a pilot test was conducted that targeted 10 people who were not medical practitioners. A group of experts collected feedback and completed the final version of the questionnaire.

The questionnaire consisted of two categories: (1) questions related to the herbal medicines usage over the past year and (2) questions related to adverse events experienced relating to the herbal medicines. The questionnaire is shown in Supplement 1 (in Supplementary Material available online at https://doi.org/10.1155/2017/4089019).

2.4. Study Variables. The detailed variables are as follows:

(1) Demographic information: sex, age, occupation, and education level

(2) Usage patterns: opinion on safety of herbal medicines, experiences related to taking herbal medicines, places from which the herbal medicines were purchased, the types of herbal medicines used, reasons for taking herbal medicines, and reasons for not taking herbal medicines

(3) Adverse events: experiences of adverse events relating to herbal medicines, types of adverse events, whether adverse events were reported, to which institutions the adverse events were reported, reasons for not reporting adverse events, how to deal with adverse events, and opinions on herbal medicines after experiencing adverse events

2.5. Statistical Analyses. A frequency analysis was performed for all variables. The chi-squared test was also employed in order to determine differences by sex, age, occupation, and education level. IBM SPSS ver. 18.0 (IBM Co., Armonk, NY, USA) was used for analysis.

2.6. Ethical Considerations. All participants were briefed with an explanation of the study's purpose prior to the initiation of the survey. Only those who voluntarily agreed to participate and to have their data collected to be published were enrolled in the study. This survey was conducted anonymously. The entire survey process was approved by the Institutional Review Board of Kyung Hee University (IRB number KHSIRB1-15-039).

3. Results

3.1. Basic Characteristics. There were total of 1,134 respondents, consisting of 591 (52.1%) men and 543 (47.9%) women. Table 1 presents the distribution of the participants' sex, age, occupation, and education level. The age distribution was as follows: 209 (18.4%) were 20–29 years old, 237 (20.9%) were 30–39 years old, 277 (24.4%) were 40–49 years old, 253 (22.3%) were 50–59 years old, and 158 (13.9%) were 60–69 years old. Office worker was the most common occupation (34.6%), and most participants (79.7%) had a university degree.

Of the 1,134 respondents, there were 693 (61.1%) who had taken herbal medicines within the past year and 441 (38.9%) who had not. There was no difference in demographic factors between users and nonusers of herbal medicines (Table 2).

3.2. Opinion on Safety of Herbal Medicines. Of the total 1,134 participants, 726 (64.0%) people responded that herbal medicine is safe and the remaining 408 (36.0%) people considered herbal medicine unsafe. Women tended to distrust the safety of herbal medicines more compared to men, and those over the age of 50 were more skeptical of herbal medicine (Table 3).

3.3. Usage Patterns of Herbal Medicines. The most common place to purchase herbal medicines was TKM hospital or clinic (63.6%). Pharmacy (17.0%), traditional herb market (17.0%), health food store (14.6%), oriental pharmacy (12.8%), home shopping (11.0%), and hypermarket

Table 1: Basic characteristics of respondents.

Demographic characteristics	*n*	%
Sex		
Men	591	52.1
Women	543	47.9
Age (years)		
20–29	209	18.4
30–39	237	20.9
40–49	277	24.4
50–59	253	22.3
60–69	158	13.9
Occupation		
Executives professionals	214	18.9
Office workers	392	34.6
Service sales workers	83	7.3
Agriculture, forestry, and fishery workers	4	0.4
Craft mechanical workers	41	3.6
Simple labourers	15	1.3
Self-employed, part-time employees, and freelancers	25	2.2
Students, housewives, and unemployed	360	31.7
Level of education		
Middle school	9	1.1
High school	218	19.2
College	784	69.1
Graduate school	120	10.6
Total	1134	100.0

(11.0%) were reported as other places to purchase them. The most predominantly used type of herbal medicines was a decoction from TKM institutions (72.2%). Other types of herbal medicines were crude herbs, which are mainly used in food or tea (35.8%), health food (28.6%), national insurance-covered herbal medicines from TKM institutions (15.3%), national insurance-covered herbal medicines from pharmacies (15.0%), and others (0.8%) (Table 4).

The reasons for taking the medication were as follows: 57.3% for "health improvement," 40.3% for "treatment in KM hospitals or clinics," 34.8% due to "recommendation from acquaintance," 9.5% due to "recommendation from a pharmacist," and others. The reasons for not taking herbal medicines were "medication was not necessary" (63.7%), "uncertainty of origins" (35.4%), "expensive prices" (25.9%), "anxiety related to the possibility of harmful substances" (25.9%), "anxiety related to the possibility of adverse events" (23.8%), and others (Table 4). Figures 1–3 present the usage patterns and reasons for taking of herbal medicines according to age groups in detail. There were no remarkable differences by age.

3.4. Adverse Events and Their Reporting. Of the 693 participants who have taken herbal medicines within the past year, 46 (6.6%) responded that they had experienced adverse events from herbal medicines. The most common symptom

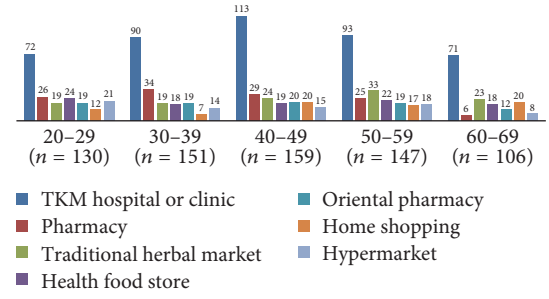

Figure 1: Location where herbal medicines were purchased according to age groups.

was digestive disorders (52.2%), followed by skin disorders (34.8%) and nervous disorders (23.9%) (Table 5).

After experiencing an adverse event, 20 participants (43.5%) were treated by KM doctors, 13 (28.5%) did not take any action, and 12 (26.1%) requested a refund. Seventeen participants (37.0%) felt that expert counselling may be needed after experiencing adverse events, and 14 (30.4%) responded that drugs can have adverse events and that they would continue taking herbal medicines. However, 13 people (28.3%) responded that they cannot trust herbal medicines anymore and they would not continue taking herbal medicines. Of the 46 respondents who experienced

Table 2: Basic characteristics of herbal medicines users and nonusers.

Demographic characteristics	Users (n = 693, 61.1%)	Nonusers (n = 441, 38.9%)	*P value
Sex			
Men	361 (52.1)	230 (52.2)	1.000
Women	332 (47.9)	211 (47.8)	
Age (years)			
20–29	130 (18.8)	79 (17.9)	
30–39	151 (21.8)	86 (19.5)	
40–49	159 (22.9)	118 (26.8)	0.221
50–59	147 (21.2)	106 (24.0)	
60–69	106 (15.3)	52 (11.8)	
Occupation			
Executives professionals	146 (21.1)	68 (15.4)	
Office workers	244 (35.2)	148 (33.6)	
Service sales workers	45 (6.5)	38 (8.6)	
Agriculture, forestry, and fishery workers	2 (0.3)	2 (0.5)	0.229
Craft mechanical workers	23 (3.3)	18 (4.1)	
Simple labourers	7 (1.0)	8 (1.8)	
Self-employed, part-time employees, and freelancers	15 (2.2)	10 (2.3)	
Students, housewives, and unemployed	211 (30.4)	149 (33.8)	
Level of education			
Middle school	6 (0.9)	6 (1.3)	
High school	119 (17.2)	99 (22.4)	0.172
College	490 (70.7)	294 (66.7)	
Graduate school	78 (11.3)	42 (9.5)	

All data are in n (%).
*Chi-square test was performed.

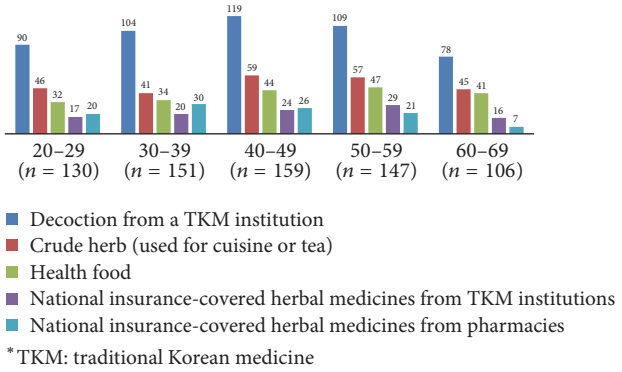

Figure 2: Types of herbal medicines according to age groups.

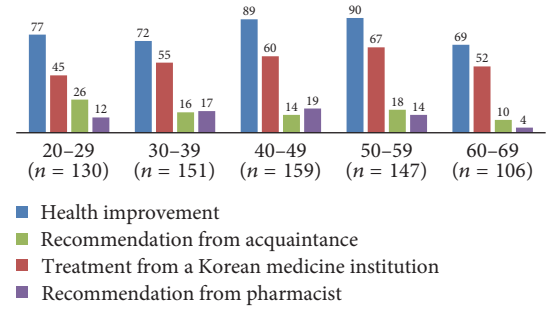

Figure 3: Reasons for taking herbal medicines according to age groups.

adverse events, 14 (30.4%) reported their adverse events and 20 (43.5%) did not because they had little information regarding to whom the report should be made (Table 6).

4. Discussion

This study described the basic characteristics of those who had taken herbal medicines and those who had not taken them, the places that herbal medicines were purchased, the reasons for taking or not taking herbal medicine, and adverse events experienced due to herbal medicines and how the adverse events were addressed. In previous studies [7, 8], elderly people tended to visit TKM institutions more frequently than younger people. However, age was not a factor that affected taking herbal medicines in this survey.

There have been several surveys in the past demonstrating consumers' opinions about the safety of herbal medicines [9–11]. According to surveys of Serbia [9] and Saudi Arabia [10], 73.3% (211) of Serbian respondents and 81.2% (239) of Saudi Arabian respondents considered that the use of herbal medicines and herbal dietary supplements is harmless, respectively. Meanwhile, only 12.1% (88) of Lebanese respondents perceived that herbal products sold in Lebanon are pure [11].

Table 3: Opinion on safety of herbal medicines.

	Herbal medicine is safe (n = 726, 64.0%)	Herbal medicine is not safe (n = 408, 36.0%)	*P value
Sex			
Men	430 (59.2)	161 (39.5)	>0.001
Women	296 (40.8)	247 (60.5)	
Age (years)			
20–29	154 (21.2)	55 (13.5)	
30–39	166 (22.9)	71 (17.4)	
40–49	176 (24.2)	101 (24.8)	>0.001
50–59	148 (20.4)	105 (25.7)	
60–69	82 (11.3)	76 (18.6)	
Occupation			
Executives professionals	145 (20.0)	69 (16.9)	
Office workers	252 (34.7)	140 (34.3)	
Service sales workers	52 (7.2)	31 (7.6)	
Agriculture, forestry, and fishery workers	4 (0.6)	0	0.142
Craft mechanical workers	32 (4.4)	9 (2.2)	
Simple labourers	11 (1.5)	4 (1.0)	
Self-employed, part-time employees, and freelancers	14 (1.9)	11 (2.7)	
Students, housewives, and unemployed	216 (29.8%)	144 (35.3)	
Level of education			
Middle school	10 (1.4)	2 (0.4)	
High school	138 (19.0)	80 (19.6)	0.610
College	499 (68.7)	285 (69.9)	
Graduate school	79 (10.9)	41 (10.0)	

All data are in n (%).
*Chi-square test was performed.

Most respondents purchased herbal medicines from TKM institutions (Table 4). This observation can be explained by the Korean health system. South Korea has adopted a dual healthcare system in which both Western medicine and TKM are permitted as legal medical care [12]. Herbal-drugs are separated from herbal supplements and are prescribed by TKM practitioners or sold over-the-counter in pharmacies. Herbal supplements, such as red ginseng, are sold in pharmacies, hypermarkets, or through home shopping channels. Traditional herb markets and health food stores generally sell crude herbs or self-made decoctions. Oriental pharmacies can provide 100 popular herbal medications without TKM practitioners' prescriptions [13].

Regarding the type of herbal medicines, a decoction from a TKM institution was the most frequently used, reflecting the preference of Koreans (Table 4). Korean people may recognise that a decoction is a typical herbal medicine that is more effective than other formulations, such as powders, pills, and capsules. On the other hand, in Japan, the proportion of the market of herbal medicines covered by insurance is large, and production costs of insured herbal medicines account for 84.2% of the entire herbal medicine market [14].

The main reasons for taking herbal medicines included "health improvement" and "treatment in TKM hospitals or clinics" (Table 4). Herbal medicine is recognised as a tool for both preventive medicine and disease treatment in South Korea. Meanwhile, traditional medicine is still one of the primary sources of health care in Africa and some other developing countries, and it is used as complementary therapy in North America and many European countries [3]. In South Korea, both uses are well-balanced thanks to the health system and cultural influences.

This study also analysed the usage patterns of difference by age (Figures 1–3). There is no noticeable outcome, though those 60–69 tend to use crude herb and health food more. It may affect their negative perception on safety of herbal medicines (Table 3). Crude herb is not purified and its safety is not proven. It also includes roots, leaves, and flowers that have been taken from the wild; accordingly, the safety of crude herb can be suspicious.

Of the 1,134 respondents, 441 (38.9%) had not taken herbal medicines in the past year (Table 2). Among the reasons for not taking herbal medicine, "uncertainty of origins," "anxiety related to the possibility of harmful substances," "anxiety related to the possibility of adverse events," and "distrust of expiry date" were due to disbelief in the safety of herbal medicines. It is necessary to improve the safety of herbal medicines in order for the herbal medicine market

Table 4: Patterns of herbal medicine use and reasons for taking or not taking herbal medicines.

Question (number of respondents)	Response	*n* (%)
Have you taken herbal medicines in the past year? (*n* = 1134)	Yes	693 (61.1)
	No	441 (38.9)
Location where herbal medicines were purchased* (*n* = 693)	TKM hospital or clinic	441 (63.6)
	Pharmacy	120 (17.0)
	Traditional herbal market	118 (17.0)
	Health food store	101 (14.6)
	Oriental pharmacy	89 (12.8)
	Home shopping	76 (11.0)
	Hypermarket	76 (11.0)
	Other	8 (1.1)
Types of herbal medicines* (*n* = 693)	Decoction from a TKM institution	500 (72.2)
	Crude herb (used for cuisine or tea)	248 (35.8)
	Health food	198 (28.6)
	National insurance-covered herbal medicines from TKM institutions	106 (15.3)
	National insurance-covered herbal medicines from pharmacies	104 (15.0)
	Other	6 (0.8)
Reasons for taking herbal medicines* (*n* = 693)	Health improvement	397 (57.3)
	Treatment from a Korean medicine institution	279 (40.3)
	Recommendation from acquaintance	84 (12.1)
	Recommendation from pharmacist	66 (9.5)
	Other	6 (0.7)
Reasons for not taking herbal medicines* (*n* = 441)	Medication not necessary	281 (63.7)
	Uncertainty of origins	156 (35.4)
	Expensive prices	114 (25.9)
	Anxiety about possible harmful substances	105 (23.8)
	Anxiety about possible adverse events	59 (13.4)
	Disbelief regarding expiry date	41 (9.3)
	No effectiveness	39 (8.8)
	Other	7 (1.6)

*Multiple responses possible; TKM: traditional Korean medicine.

to grow. In South Korea, regulations on manufacturing and quality control of herbal medications were established in 2012, and they became fully mandatory in 2015 [15]. Health food is subject to the Good Manufacturing Practices (GMP) of the Ministry of Food and Drug Safety (MFDS) [16]. However, there is no safety control system for herbal medicines distributed via other routes. In Japan, unlike in South Korea, herbal medicines are divided into 210 over-the-counter Kampo products, crude drugs, and Kampo extracts and Western traditional herbal products, which are separately managed according to the national system [17].

Adverse events from herbal medicines reported by 46 participants (6.6% of herbal medicines users) primarily included digestive, skin, and nervous disorders (Table 5). Liver toxicity of herbal medicines is controversial when it comes to the safety of herbal medicine [18]. However, only 4 cases of liver disorders were reported among the total 77 cases. According to previous studies [6, 19], the most frequently reported adverse drug reactions in a single hospital were gastrointestinal disorders and skin reactions, similar to the results in our study.

Although the Korean adverse drug reaction surveillance system was established in 1988 [20], it is not appropriate for reporting adverse events due to herbal medicines. Since 2012, adverse reactions from approved herbal-drugs have been reported to the Korea Adverse Event Reporting System (KAERS). However, not every herbal medicine is registered in that system, and the formulation of decoctions has not been applicable. The problem about national pharmacovigilance system of South Korea was also raised in prior study [21]. On

Table 5: The number of adverse events due to herbal medicines.

Question (number of respondents)	Response	*n* (%)
Have you experienced adverse events from herbal medicines in the past year? (*n* = 693)	Yes	46 (6.6)
	No	647 (93.4)
What types of adverse events have you experienced?* (*n* = 46)	Digestive system	24 (52.2)
	Skin	16 (34.8)
	Nervous disorder	11 (23.9)
	Systemic disorder	6 (13.0)
	Liver	4 (8.7)
	ENT and eye	3 (6.5)
	Cardiovascular system	3 (6.5)
	Circulatory system	2 (4.3)
	Kidney	2 (4.3)
	Urinary system	2 (4.3)
	Musculoskeletal system	2 (4.3)
	Respiratory system	2 (4.3)

*Multiple responses possible; ENT, ear, nose, and throat.

Table 6: Behaviours and opinions related to herbal medicine after experiencing adverse events.

Question (number of respondents)	Response	*n* (%)
Did you report adverse events? (*n* = 46)	Yes	14 (30.4)
	No	32 (69.6)
To whom did you report adverse events?* (*n* = 14)	TKM institution	10 (71.4)
	WM institution	3 (21.4)
	Pharmacy	3 (21.4)
	Public health centre	2 (14.3)
	Ministry of Health and Welfare	2 (14.3)
	Korea Consumer Agency	1 (7.1)
	KIDS	1 (7.1)
	MFDS	1 (7.1)
Why did you not report adverse events? (*n* = 32)	I did not know where to report	20 (62.5)
	I felt it was unnecessary	7 (21.9)
	I felt lazy	5 (15.6)
How did you deal with adverse events?* (*n* = 46)	Consulted with TKM doctors	20 (43.5)
	Nothing specific	13 (28.3)
	Requested refund	12 (26.1)
	Consulted with WM doctors	7 (15.2)
	Consulted with pharmacist	3 (6.5)
What did you think after the adverse events? (*n* = 46)	I need to see an expert	17 (37.0)
	Drugs can have adverse events and I am going to continue taking herbal medicines	14 (30.4)
	I cannot trust herbal medicines anymore and I am not going to take herbal medicines	13 (28.3)
	I do not know	2 (4.3)

*Multiple responses possible. TKM: traditional Korean medicine; WM: western medicine; KIDS: Korea Institute of Drug Safety and Risk Management; MFDS: Ministry of Food and Drug Safety.

the other hand, in Taiwan, the compositions and formulations of herbal medicines are included when reporting adverse drug reactions [22].

After experiencing adverse events, the majority of respondents (20; 43.5% of adverse event experiencers) visited TKM practitioners, and 17 (37.0%) felt that expert counsel would be necessary (Table 6). These results suggest that the role of TKM practitioners is important when adverse events occur. Moreover, only 14 people (30.4% of adverse events experiencers) reported their adverse events, and only one person reported it to the Korea Institute of Drug Safety and Risk Management (KIDS) correctly. Therefore, both TKM practitioners and consumers need to receive appropriate education for responding to adverse events due to herbal medicines. Additionally, the reliability on herbal medicines was reduced for 13 respondents after they experienced adverse events (28.3% of adverse events experiencers), and they responded that they would not take any other herbal medicines. Such behaviours may lead to a decreased consumption of herbal medicines; consequently, the safety of herbal medicines is vitally important.

There are limitations of this survey study. Firstly, a recall bias may exist because this study was based on a retrospective survey. Secondly, there is a possibility of response bias because the participants are rather highly educated. This is because having recruited the participants through an online research company and the sample may not be representative of the general population. Lastly, the perception of the range of herbal medicines varies among Koreans. Some people only recognise herbal medicines from TKM institutions as herbal medicines, while others take into account every type of herb.

Nonetheless, this study is meaningful in that there are no previous surveys to date that systematically investigated experiences and opinions about herbal medicines. This survey, unlike other consumer surveys, included not only herbal medicines users but also nonusers as participants, which increased the representativeness of the general population.

5. Conclusions

This survey analysed the usage of herbal and medicinal products in South Korea. This study showed the demographic differences between herbal medicine users and nonusers, opinions on safety of herbal medicines, experiences of using herbal medicines, and adverse events experienced from using herbal medicines. The major reasons for not taking herbal medicines were based on a disbelief in their safety. Therefore, it is important to ensure not only the efficacy but also the safety of herbal medicines in order to expand herbal product markets. Specific regulations on herbal medicines are needed to resolve problems with their origins, possibility of containing harmful substances, and the expiry date.

Abbreviations

WHO: World Health Organization
T&CM: Traditional and Complementary Medicine
TKM: Traditional Korean medicine
GMP: Good Manufacturing Practice
MFDS: Ministry of Food and Drug Safety
KAERS: Korea Adverse Event Reporting System
KIDS: Korea Institute of Drug Safety and Risk Management.

Ethical Approval

This survey was approved by Institutional Review Board of Kyung Hee University (IRB no. KHSIRB1-15-039).

Consent

This study did not contain any individual person's data; however, it was notified that collected data would be published. All participants voluntarily agreed to participate in this survey.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Soobin Jang and Kyeong Han Kim drafted the manuscript. Eun-Kyung Lee and Seung-Ho Sun managed entire process of survey and extracted the data. Bo-Hyoung Jang and Ho-Yeon Go organized and conducted the study. Yong-Cheol Shin and Seong-Gyu Ko supervised the study. All authors read and approved the final manuscript.

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References

[1] T. K. K. Smith, V. Eckl, and J. Johnson, "Sales of herbal dietary supplements in US increased 7.5% in 2015 consumers spent $6.92 billion on herbal supplements in 2015, marking the 12th consecutive year of growth," *HerbalGram*, vol. 111, pp. 67–73, 2016.

[2] T. Citarasu, "Herbal biomedicines: a new opportunity for aquaculture industry," *Aquaculture International*, vol. 18, no. 3, pp. 403–414, 2010.

[3] World Health Organization, *WHO Traditional Medicine Strategy 2014–2023*, World Health Organization, Geneva, Switzerland, 2013.

[4] Ministry of Health and Welfare, *Report on the Usage and Consumption of Korean Medicine (2011)*, 2011.

[5] S. Y. Ha and K. W. Kang, "Two cases of lead poisoning after taking herb pills hwangdan (red lead)," *Korean Journal of Pediatrics*, vol. 22, no. 1, pp. 64–70, 1979.

[6] Y. J. Kwon, W. K. Cho, and C. H. Han, "Status of herbal-drug-associated adverse drug reactions voluntarily reported by EMR," *The Journal of Korean Oriental Internal Medicine*, vol. 33, no. 4, pp. 485–497, 2012.

[7] J. Yoon, H. Park, C. Chu, S.-Y. Choi, K. Lee, and S. Lee, "A study on the characteristics of infrequent and frequent outpatients visiting Korean traditional medical facilities," *Osong Public Health and Research Perspectives*, vol. 6, no. 3, pp. 170–183, 2015.

[8] J.-M. Woo, E.-J. Park, M. Lee, M. Ahn, S. Kwon, and K. H. Koo, "Changes in attitudes toward and patterns in traditional Korean medicine among the general population in South Korea: a comparison between 2008 and 2011," *BMC Complementary and Alternative Medicine*, vol. 14, article 436, 2014.

[9] I. Samojlik, V. Mijatović, N. Gavarić, S. Krstin, and B. Božin, "Consumers' attitude towards the use and safety of herbal medicines and herbal dietary supplements in Serbia," *International Journal of Clinical Pharmacy*, vol. 35, no. 5, pp. 835–840, 2013.

[10] A. K Suleiman, "Attitudes and beliefs of consumers of herbal medicines in Riyadh, Saudi Arabia," *Journal of Community Medicine & Health Education*, vol. 4, article 269, 2014.

[11] G. El Khoury, W. Ramadan, and N. Zeeni, "Herbal products and dietary supplements: a cross-sectional survey of use, attitudes, and knowledge among the Lebanese population," *Journal of Community Health*, vol. 41, no. 3, pp. 566–573, 2016.

[12] H. Lee, "The globalization of East Asian traditional medicine and the change of medical hegemony," *Society and Theory*, vol. 22, no. 5, pp. 377–398, 2013.

[13] Pharmaceutical Affairs Law (Republic of Korea) article 23, http://www.law.go.kr/lsInfoP.do?lsiSeq=178173&efYd=20161230#0000.

[14] Japanese Kampo Medicines Manufactures Association, *Annual Report on Pharmaceutical Industry Production Statistics*, 2014.

[15] Ministry of Food and Drug Safety, *Guideline on Good Manufacturing Practice of Herbal Medicines*, 2013.

[16] Ministry of Food and Drug Safety, http://www.mfds.go.kr.

[17] H. Maegawa, T. Nakamura, and K. Saito, "Regulation of traditional herbal medicinal products in Japan," *Journal of Ethnopharmacology*, vol. 158, part B, pp. 511–515, 2014.

[18] Y. J. Yun, B. C. Shin, M. S. Lee, S. I. Cho, J. H. Park, and H. J. Lee, "Systematic review of the cause of drug-induced liver injuries in Korean literature," *Journal of Korean Medicine*, vol. 30, no. 2, pp. 30–45, 2009.

[19] M. Kim and C. Han, "Analysis of herbal-drug-associated adverse drug reactions using data from spontaneous reporting system in electronic medical records," *Journal of Korean Medicine*, vol. 36, no. 1, pp. 45–60, 2015.

[20] N.-K. Choi and B.-J. Park, "Adverse drug reaction surveillance system in Korea," *Journal of Preventive Medicine and Public Health*, vol. 40, no. 4, pp. 278–284, 2007.

[21] H.-K. Shin, S.-J. Jeong, D. S. Huang, B.-K. Kang, and M. S. Lee, "Usage patterns and adverse experiences in traditional Korean medicine: results of a survey in South Korea," *BMC Complementary and Alternative Medicine*, vol. 13, article 340, 2013.

[22] Taiwanese Ministry of Health and Welfare, http://www.mohw.gov.tw/cht/DOCMAP/DM1.aspx?f_list_no=205.

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Research Article

Distribution of Mast Cells and Locations, Depths, and Sizes of the Putative Acupoints CV 8 and KI 16

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The anatomical locations and sizes of acupuncture points (APs) are identified in traditional Chinese medicine by using the cun measurement method. More precise knowledge of those locations and sizes to submillimeter precision, along with their cytological characterizations, would provide significant contributions both to scientific investigations and to precise control of the practice of acupuncture. Over recent decades, researchers have come to realize that APs in the skin of rats and humans have more mast cells (MCs) than neighboring nonacupoints. In this work, the distribution of MCs in the ventral skin of mice was studied so that it could be used to infer the locations, depths from the epidermis, and sizes of three putative APs. The umbilicus was taken as the reference point, and a transversal cross section through it was studied. The harvested skins from 8-week-old mice were stained with toluidine blue, and the MCs were recognized by their red-purple stains and their metachromatic granules. The three putative APs, CV 8 and the left and the right KI 16 APs, were identified based on their high densities of MCs. These findings also imply that acupuncture may stimulate, through MCs, an immune response to allergic inflammation.

1. Introduction

Acupuncture has been a major medical practice for thousands of years in China, Korea, and Japan, yet the mechanism underlying acupuncture has still not been unambiguously identified on a scientific basis and, thus, needs to be investigated further. Among the findings of numerous research efforts that have investigated the mechanism underlying acupuncture, the finding that acupuncture treatment appears to improve the immune function seems particularly notable [1]. Mast cells (MCs) are active carriers of innate immunity against such conditions as allergies and inflammatory diseases [2, 3]. Therefore, the finding that the population of MCs is denser at APs and meridians than at nearby nonacupoints is not surprising [4, 5]; stimulation of the APs by using electroacupuncture or moxibustion has been found to induce degranulation of MCs [6–8]. This cytological characteristic of APs is in accordance with the recent finding that a particular tissue, called the primo node, in the primo vascular system (PVS) has a high population density of MCs [9]. The PVS was first proposed as the anatomical structure corresponding to the APs and meridians by BH Kim in the early 1960s, and it has long been thought, but not proven, to be a possible scientific foundation for Korean medicine [10].

In traditional Chinese medicine, the anatomical locations of APs can be identified by using the cun measurement method. However, dermal electrical impedance measurements do not significantly improve the precisions of those

locations, and information on the depths from the epidermis and the sizes of the APs is totally lacking. Thus, in this work, we used the distributions of the MCs at three acupoints, the CV 8 and the left and the right KI 16 APs, to estimate their locations, sizes, and depths from the epidermis. Knowledge of those parameters to submillimeter precision, along with knowledge of their cytological characteristics, would provide significant contributions to scientific investigations and to precise control of the practice of acupuncture.

Apart from acupuncture and the immune response, MCs synthesize, store, and release histamine and other mediators of inflammation [11, 12] and are crucial for the maintenance of tissue homeostasis, tissue repair, and the remodeling of the extracellular matrix [13, 14]. In addition, MCs accelerate epithelial-to-mesenchymal transitions, extracellular matrix degradation, and disease progression in some carcinomas [15]. Therefore, MCs are important cytological ingredients connecting Western and Eastern medicine [16].

In Western medicine, the locations and the distributions of MCs have been studied in connection with their physiological functions. For instance, MCs are located at the host-environment interface so as to initiate the host's defenses against intruders [17]. An early quantitative study on the distribution of MCs in normal mouse skin was performed by Larsson and Sylven to address the reactions of MCs to different chemical agents [18]. They developed a fairly reliable counting technique, but their study was limited to a small area of the dorsal skin, that is, an area on each side of the spine in the interscapular regions. They found that the numbers of MCs showed left-right symmetry in each individual, but the variations in the numbers of both dermal and hypodermal MCs between individuals were so large that the determination of an average standard number was not practicable. The MCs of the skin lie in the dermis and the hypodermis, where they are grouped around blood vessels and nerves. A quantitative study on the association between MCs and blood vessels in human skin was first reported by Eady et al. [19]. They observed statistically significant correlations between MC counts and blood vessel counts in skin from the upper arm, but no similar correlations were observed in skin from the forearm. They also confirmed an uneven distribution of dermal MCs in human skin, which was consistent with the previous finding for mouse skin.

Although researchers in Western medicine noticed a highly-clumped distribution of dermal MCs with a population density that varied greatly within a small area encompassing a few mm^2 of skin [19], they did not recognize the relation between the regions of the skin with the clumped high density of MCs and the APs. Zhu et al. pointed out that higher densities of MCs were found at some APs compared with neighboring nonacupoints [4, 5]. MCs, blood vessels, and nerves were found to gather to form a complex at APs, and acupuncture stimuli were found to cause MCs to migrate to and be recruited in the APs and the meridians [20]. However, they compared the densities of MCs in the APs with those of unspecified nonacupoints rather than showing the distribution of MCs throughout the skin. Therefore, the question of whether the densities of MCs in the skin could be used to get information on the locations, depths, and sizes of APs was not answered. Independently, the evidence for higher densities of MCs at APs was reinforced by a series of works on the abundance of MCs in the PVS in the abdominal cavity [21–23] and the abdominal wall [9]. This PVS prompted us to develop the current scheme of estimating the anatomical parameters for the APs in skin by making use of the distribution of MCs. Furthermore, a potential application of this anatomical information has already been proposed: the monitoring of the behaviors of APs during acupuncture treatment by measuring electroactive molecules, such as serotonin, secreted by granules of MCs [24].

Because of the significant roles of MCs in both Western medicine and acupuncture, systematic investigations of the characteristics of their distributions in skin so as to extend the findings in previous works would be desirable [4, 5, 18, 19]. For this purpose, the number of MCs in a cross section of skin needs to be determined for the various APs. In this work, we examined a cross section of the abdominal wall. When we tried to observe a sectional view including several APs, we had to be certain not to miss the APs. We found that the APs could easily be missed because the precisions of the locations of the APs were only defined on the order of millimeters in terms of the F-cun method [25]; such a precision was too coarse for histological work involving sections with thicknesses of 5 to 10 μm. Because the umbilicus is one of the most well-defined points in the gross anatomy of abdominal skin and is known as conception vessel 8 (Shenque, CV 8), we chose it as the reference point for our histological work. As shown in Figure 1, two other APs, the kidney 16 (Huangshu, KI 16) and the stomach 25 (Tianshu, ST 25) APs, are located at half F-cun and two F-cun, respectively, from CV 8 along the transversal line. Consequently, for our study, we chose a transverse section of the abdominal wall across the CV 8 acupoint.

2. Materials and Methods

2.1. Animal Preparation and Sample Taking. For this investigation, 10 male 8-week-old ICR mice were obtained from Young Bio (Seoul, Korea). They were kept in an air-conditioned room at constant temperature and relative humidity (23°C and 60%, resp.) with a 12-hour/12-hour natural light/dark cycle and ad libitum access to food and water. The animals were handled according to current international laws and policies (*Guide for the Care and Use of Laboratory Animals*, National Academy Press, 1996), and the care of the animals and the procedures used in this research were approved by the Institutional Ethics Committee of the Advanced Institute of Convergence Technology, Seoul National University (approval number: WJIACUC20160722-3-01).

The mice were anesthetized by using an intramuscular injection of a regimen consisting of 1.5 g/kg urethane and 20 mg/mL xylazine. The volume of anesthesia administered to the mice was 0.04 mL. All surgery was performed under deep anesthesia, and every effort was made to minimize suffering. The mice were sacrificed by overanesthetizing without any perfusion.

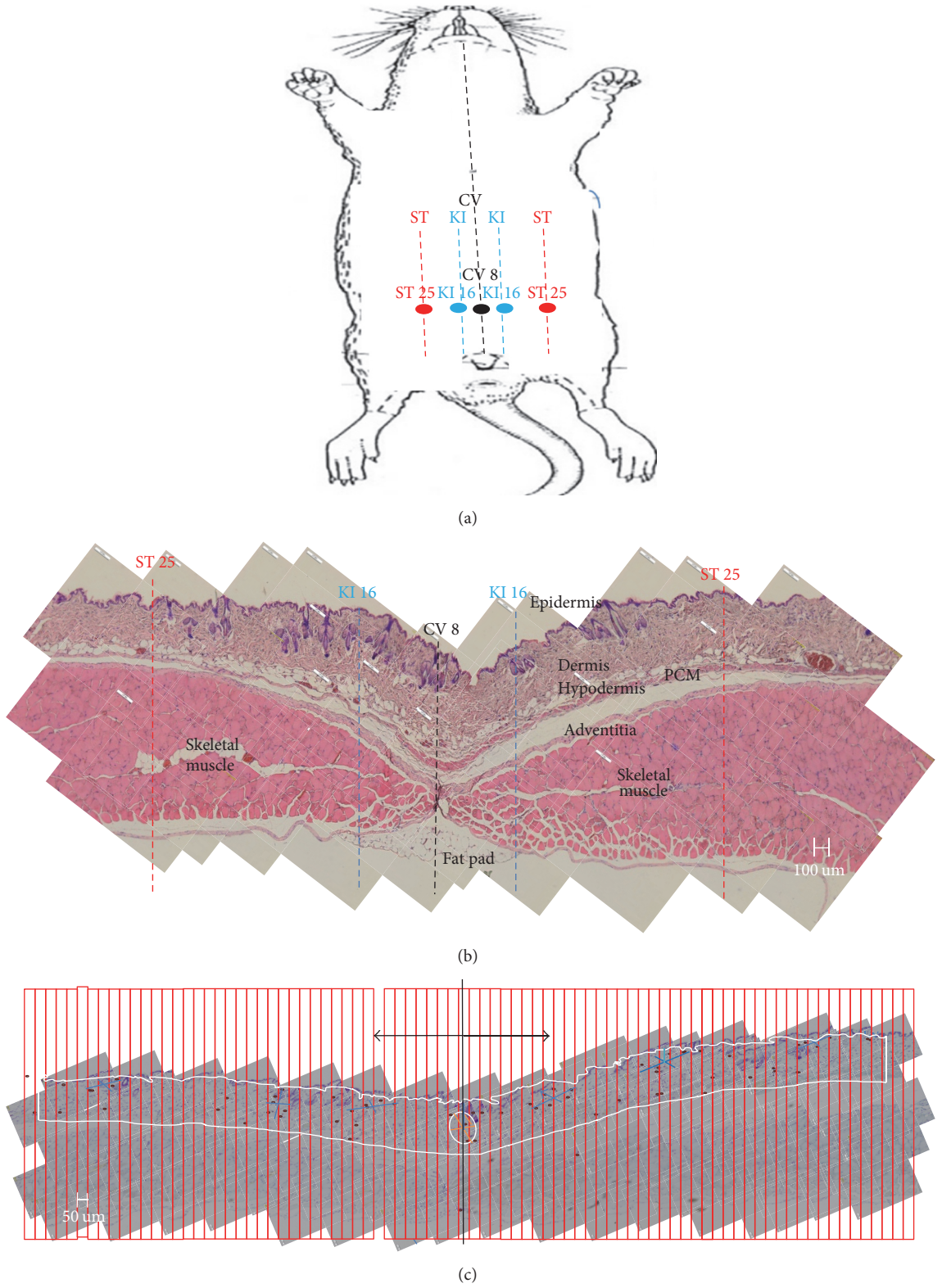


Figure 1: (a) Schematic illustration of acupoints along the meridian in the abdomen of a mouse. Three meridians, conception vessel (CV), kidney (KI) lines, and stomach (ST) lines, are shown. The acupoint CV 8 is located at the umbilicus. (b) Cross-sectional image of a mouse abdomen showing its layers (epidermis, dermis, hypodermis, panniculus carnosus muscle (PCM), adventitia, skeletal muscle, fascia, and fat pad). The traditional locations of acupoints are indicated with dotted lines. (c) A toluidine-blue stained sample was imposed by a mesh of strips (50 μm each) in order to facilitate counting of MCs. The acupoint CV 8 is indicated with an ellipse and the region of background is shown by the enclosing curve.

The anterior abdominal wall was removed from the mouse through an incision from immediately below the xiphoid cartilage to the bottom line of the urinary bladder so as to include either side of the right and the left superficial epigastric vessels bilaterally. We removed the specimen and washed it for an hour in tap water, after which it was immediately placed in a 10% neutral buffered formalin (NBF) solution and stored for one day at room temperature. We also used a second fixative called Orth's solution, which contains potassium dichromate. In this procedure, after Orth's fixation for a minimum of 24 hours, the specimens had to be stored in 70% ethyl alcohol (EtOH) until until the surface of the specimen showed any signs of the Orth-brown pigment.

After the fixation steps, we used a razor blade to remove the skin sample from a region ranging from about 500 μm above to 500 μm below the umbilicus and sectioned it transversally to permit wider access to the regions of the CV 8 and the right and the left KI 16 APs bilaterally. These acupuncture points were determined based on the traditional finger-cun measurement procedure corresponding to that for humans. All observations and operations were performed under a stereomicroscope (SZX12, Olympus, Japan).

2.2. Staining. In the first fixing method, the isolated abdominal wall specimens were fixed immediately with NBF at 23°C for 24 hr ± 5 hr. The specimens gathered were processed in an automated tissue processor, after which they were embedded in paraffin wax. The resulting formalin-fixed paraffin-embedded blocks were cut into 5 μm thick sections by using a microtome (Reichert Jung 820, Leica, Germany). The sectioning continued until the search area, the middle parts of the umbilicus region (CV 8), had been reached. In the second fixing method, the isolated abdominal wall samples were immediately fixed with Orth's fixation solution at 23°C for 24 hours while avoiding exposure to light. After fixation, the samples were washed in running water overnight and were then stored in 70% EtOH until use.

Two consecutive paraffin sections of 5 microns each from the search point were cut from the paraffin block. One was stained using the conventional hematoxylin and eosin (H&E) staining method and the other section was stained using the toluidine-blue staining method. We performed the H&E staining following a conventional procedure for the purpose of identifying the general histological features of the specimen. For the toluidine-blue staining, the toluidine-blue stock solution was made by melting 0.1 gm of toluidine-blue powder (toluidine-blue O, 198161-5G, Sigma-Aldrich, St. Louis, MO, USA) and 10 mL of 70% alcohol. The working solution at pH 2.3 was made by mixing the stock solution with sodium chloride (1%, pH 2.3). The specimens were stained with toluidine-blue for 60 ± 20 sec. They were dehydrated by dipping them quickly 10 to 15 times first in 95% ethanol and then in 100% ethanol. They were then dipped in xylene for 3 minutes, after which they were mounted on a glass slide.

2.3. Counting of Mast Cells

2.3.1. Finding the High-Density Area. The stained specimens were observed under a phase contrast microscope (BX51, Olympus, Japan) to count the MCs, which were easily recognizable because of their stained red-purple (metachromatic staining) color and the background's blue color. The granules from the MCs, which were often scattered around the MCs, were a prominent signature of the MCs. As described by earlier researchers, the distribution of dermal MCs was uneven.

A mechanical calculation of the density of mast cells in dermal connective tissue could have easily led to an erroneous value because of various deformations of the skin, such as shrinkage and distortion, which occurred during the preparation of the sample [18, 19]. Therefore, the counting had to be defined specifically to fit the purpose of the work. In the present work, we divided the abdominal wall into three layers, as shown in Figure 1(c). The 1st layer was the skin from the epidermis to the adventitia of the skeletal muscle. That layer included the dermis and the hypodermis, and the MCs were found to populate this skin layer mostly. The 2nd layer was from the adventitia to the fascia underneath the abdominal muscle. The 3rd layer was the fat tissue below the muscle layer. The fat tissue formed a pad directly below the umbilicus; MCs were also observed in that pad.

MC counts per length, instead of area, were considered by Larsson and Sylven as a practical way of density comparison [18], but we modified that method to apply a mesh of 50 μm strips to the sample, as shown in Figure 1(c). These narrow strips helped experimenters to count the MCs correctly without missing any or overcounting them. The MC counts of two consecutive 50 μm strips were recorded and presented graphically in Figure 2(a) in the form of histograms.

2.3.2. Calculating the MC Number Density at the APs in the Skin and for the Background. Having approximately found the high-density regions by using the histograms, we calculated more precisely the number density of MCs by measuring the area of each region by drawing, as shown in Figure 2(b), an ellipse that covered the MCs. Instead of the density at nonacupoints, the overall background average density throughout the entire skin of the dermis and the hypodermis, excluding the high-density regions, was calculated. For this, we used ImageJ and the TSView programs and counted the numbers of pixels. The size of a pixel was $0.64 \times 0.64\ \mu m^2$. The numbers of MCs, the areas of the high-density regions, and the number densities are presented in Table 1. The average density and other quantitative data were expressed as means ± standard deviations for ten animals. The locations of the putative APs, the CV 8 and the left and the right KI 16 APs, as determined from the densities of MCs, were given as the centers of the ellipses that covered the high-density areas. The locations, depths, and sizes of these putative APs are presented in Table 2. The average was taken over ten mice, and the standard deviations of the error are given.

3. Results

MCs were mostly distributed in the dermis and the hypodermis but were never found in the epidermis. Only very few MCs were observed near blood vessels below the hypodermis, that is, in the layers of the adventitia, skeletal muscle, and

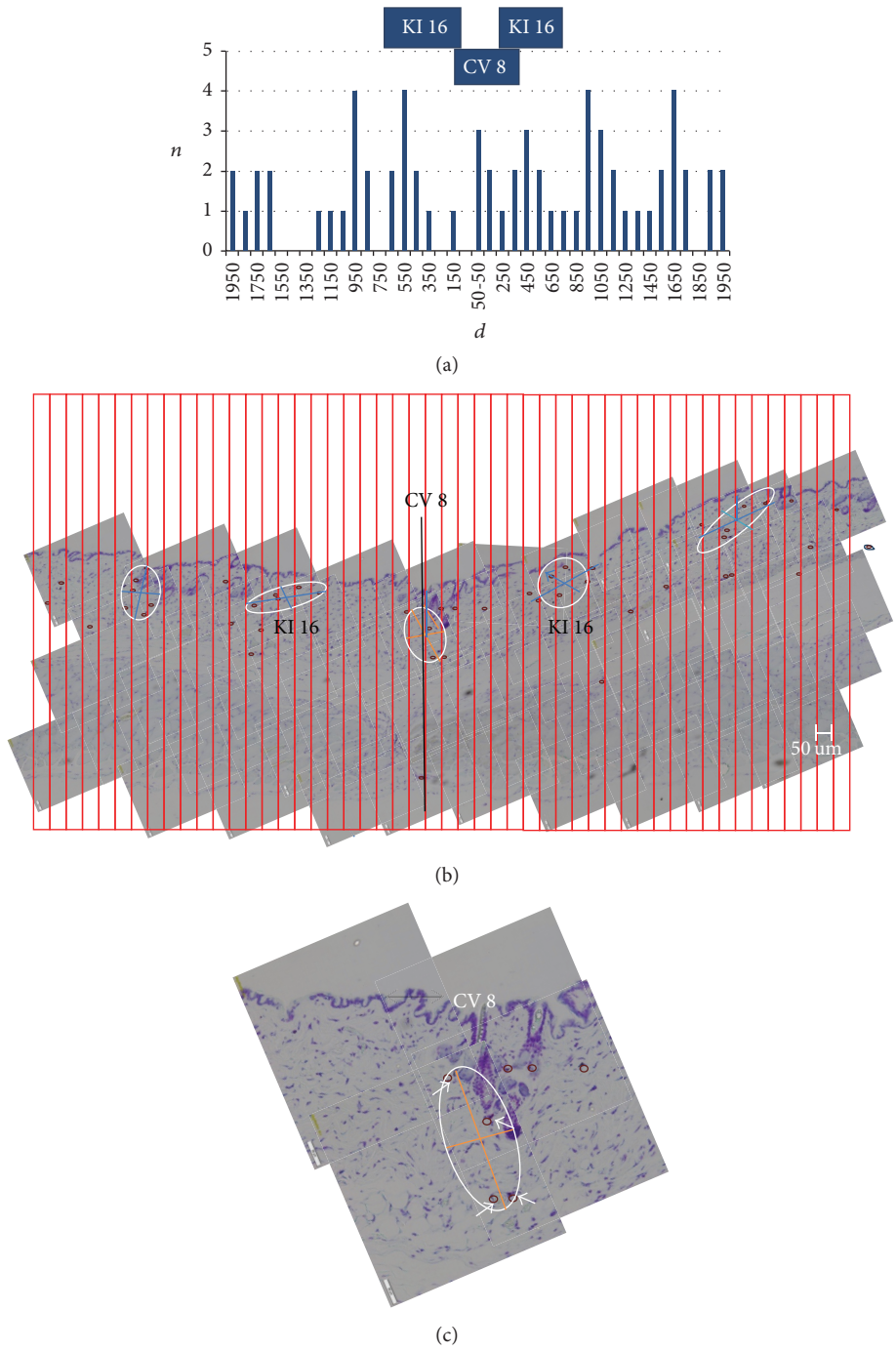


Figure 2: (a) Graphical representation of MC density from Figure 1(c). X-axis (d) is the distance from CV8 in unit of μm. Y axis (n) is the number of MCs in 100 μm. (b) The same image of Figure 1(c) showing the selected five regions of high density of MCs with ellipses. The putative acupoints CV 8 and right and left KI 16 are indicated. Two more high-density points are also shown. They might correspond to ST 25 s. (c) A magnified view of the region of CV 8 in Figure 1(c). Four MCs are indicated with arrows. The center and the long and short axes of the covering ellipse are location and size of CV 8, respectively. The center lied in deep dermis below a hair follicle.

fascia. Surprisingly, MCs again occurred in the fat pad underneath the fascia, a finding that was not reported in previous studies [18, 19]. From the graphs in Figure 2(a) showing the MC distributions, one can clearly see that the distributions are not even. From this gross feature of the distributions we selected regions that should be examined more closely. Figure 2(b) shows the five selected regions of abundant MCs.

We examined the locations of the high-density regions in connection with the three APs, the CV 8 and the right and the left KI 16 APs. In Table 1, we summarize data for the MC densities at the three putative APs and for the background.

Table 1: Number density of mast cells at the putative acupoints CV 8 and KI 16 and at the fat pad and the background density.

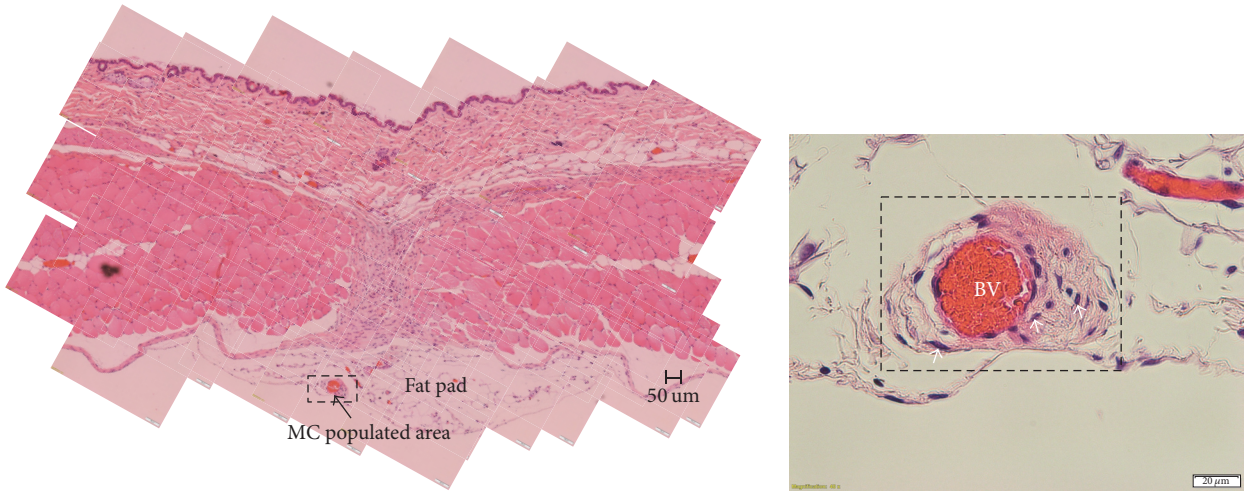
M	A/W	CV 8			Right KI 16			Left KI 16			Right NHP			Left NHP			B			Fat pad		
		N	A	D	N	A	D	N	A	D	N	A	D	N	A	D	N	A	D	N	A	D
1	8/26	4	2.6	1.5	7	1.8	3.9	5	2.3	2.2	6	1.9	3.2	6	2.7	2.2	33	83.1	0.1	1	2.5	0.4
2	8/25	4	1.4	2.9	5	1.8	2.8	3	1.4	2.1	6	2.3	2.6	3	2.7	1.4	28	278.6	0.1	2	33.3	0.1
3	8/25	2	0.7	2.9	3	1.4	2.1	4	1.3	3.1	4	1.6	2.5	6	1.4	4.3	30	132.1	0.2	7	13.8	0.5
4	8/26	10	1.3	7.7	4	2.1	1.9	3	0.9	3.3	4	2	2	4	1.4	2.9	40	81.7	0.5	5	8.6	0.6
5	8/26	2	0.9	2.2	4	1.2	3.3	2	0.6	3.3	6	1.3	4.6	3	1.1	2.7	43	153.8	0.3	4	9.8	0.4
6	8/27	3	1.8	1.7	3	3.5	0.9	4	3.1	1.3	4	3	1.3	4	4	1	33	105.1	0.3	5	26.1	0.2
7	8/24	5	1.7	2.9	3	1	3	7	2.7	2.6	3	1.8	1.7	3	5.6	0.5	26	119.6	0.2	1	0.8	1.3
8	8/27	2	0.5	4	2	0.5	4	2	0.8	2.5	4	1.0	4	5	3.9	1.3	5	99.2	0.1	3	10.3	0.3
9	8/26	3	0.8	3.8	2	0.7	2.9	5	1	5	4	1.6	2.5	7	1.6	4.4	37	162.8	0.2	4	9	0.4
10	8/24	2	0.6	3.3	4	1.7	2.4	4	1.3	3.1	5	2.2	2.3	3	1.7	1.8	40	76.2	0.5	6	3.5	1.7
Average	8/25.6	3.7	1.2	3.3	3.7	1.6	2.7	3.9	1.5	2.9	4.6	1.9	2.7	4.4	2.6	2.3	31.5	129.2	0.3	3.8	11.8	0.6
SD	0/1.0	2.3	0.7	1.7	1.4	0.8	0.9	1.4	0.8	0.9	1.0	0.5	1.0	1.4	1.5	1.3	10.3	57.3	0.1	1.9	9.9	0.5

M = mouse number; A/W = age (week)/weight (g); NHP = next high MC density point; B = background; N = number of MCs; A = area (10^4 um^2); $D = N/A$ (number/10^4 μm).

Table 2: Locations, depths, and sizes of the putative acupoints CV 8 and KI 16.

M	A/W	CV 8			Right KI 16			Left KI 16			Right NHP			Left NHP		
		X_c	Z	L/S	X	Z	L/S	X	Z	L/S	X	Z	L/S	X	Z	L/S
1	8/26	+114	250	283/117	416	177	208/113	443	88	245/119	964	133	233/105	883	164	293/118
2	8/25	−50	182	152/117	783	113	233/96	712	79	154/115	1467	383	258/115	1430	85	163/163
3	8/25	+17	25	128/67	450	129	203/89	550	194	167/100	1190	325	207/99	1767	258	144/128
4	8/26	+175	50	195/85	588	183	165/165	683	283	143/80	1523	250	218/119	1126	300	236/77
5	8/26	−67	133	133/83	356	64	180/82	318	137	90/86	922	112	149/111	1393	58	123/114
6	8/27	+50	317	228/98	483	233	275/163	580	67	220/179	1445	70	285/132	1617	233	250/202
7	8/24	+25	153	238/92	673	222	175/76	771	219	254/133	1634	171	174/132	1445	219	320/222
8	8/27	−44	290	116/60	888	220	83/83	738	111	137/73	1206	102	130/101	1208	109	265/187
9	8/26	−67	290	105/94	525	233	100/83	209	62	142/90	1188	112	208/99	939	56	183/110
10	8/24	+100	246	88/87	256	60	167/133	650	117	179/95	783	167	230/123	1251	200	214/102
Average	8/25.6	70.9	193.6	157.9/90	541.8	163.4	178.9/108.3	565.4	135.7	173.1/107	1232.2	182.5	209.2/113.6	1305.9	168.2	219.1/142.3
SD	0/1.0	44.9	97.0	62.1/17.5	184.8	64.2	54.2/32.2	178.4	69.7	49.4/29.8	268.5	98.5	45.1/12.1	266.5	82.7	61.9/45.7

M = mouse number; A/W = age (week)/weight (g).
X_c = distance from the navel (μm) in CV 8; X = distance from CV 8 to KI 16 acupoints and NHPs, respectively.
Z = depth from the epidermis (μm).
L/S = long axis/short axis of the covering ellipse (μm).



(a) (b)

Figure 3: (a) An H&E image showing the fat pad under the abdominal wall muscle. The MC populated region is indicated with a box. (b) A magnified view of the boxed region of (a). Three MCs near a blood vessel (BV) are indicated with arrows.

The average MC number densities were $3.3 \pm 1.7/(100\,\mu m)^2$, $2.7 \pm 0.9/(100\,\mu m)^2$, and $2.9 \pm 0.9/(100\,\mu m)^2$ at the CV 8 and the right and the left KI 16 acupoints, respectively, where we have used the practical and convenient unit of area for experimenters, that is, $100\,\mu m \times 100\,\mu m$. The MC number density of the background was $0.3 \pm 0.1/(100\,\mu m)^2$. The area and the MC density at the fat pad were $11.8 \pm 9.9 \times 10^4\,\mu m^2$ and $0.6 \pm 0.5/(100\,\mu m)^2$, the latter being about two times higher than the background density of the dermis and the hypodermis.

In Table 2, the locations, that is, the distances from the umbilicus, the depths from the epidermis, and the sizes of the long and the short axes of the three APs are recorded. The distances from the CV 8 to the right and the left KI 16 APs were $541.8 \pm 184.8\,\mu m$ and $565.4 \pm 178.4\,\mu m$, respectively. The depths were $193.6 \pm 97.0\,\mu m$, $163.4 \pm 64.2\,\mu m$, and $135.7 \pm 69.7\,\mu m$ for the CV 8 and the right and the left KI 16 APs, respectively. The centers of the APs were mostly in the deep dermis and sometimes in the upper hypodermis. The average long axis × the short axis for the CV 8 AP was $157.9 \pm 62.1\,\mu m \times 90.0 \pm 17.5\,\mu m$. Similarly, for the right and the left KI 16 APs, they were $178.9 \pm 54.2\,\mu m \times 108.3 \pm 32.2\,\mu m$ and $173.1 \pm 49.4 \times 107.0 \pm 29.8\,\mu m$, respectively.

The region corresponding to the CV 8 AP is shown in Figure 2(c). The area and the MC count in it were $2.6 \times 10^4\,\mu m^2$ and 4, respectively, giving a number density of $1.5/(100\,\mu m)^2$. This region was enclosed by an ellipse with long and short axes of $283\,\mu m$ and $117\,\mu m$, respectively. This can be considered as a gross anatomical description of the putative CV 8 AP.

Figure 3 shows a fat pad with a diameter. MCs were observed near the blood vessels in this fat pad. However, MCs of other mice were not in the similar location, sometimes being near the fascia and other times being near the parietal peritoneum. The distribution was not left-right symmetric either.

4. Discussion

Previous studies [4–8] on the relation between MCs and APs considered traditionally defined APS and they found higher densities of MCs at APs than neighboring non-APs. However, they did not investigate whether only APs have many MCs or there are non-APs which still have high density of MCs. Logically speaking, they found that the high MC density is a necessary condition of traditional APs but did not know whether it is also a sufficient condition. In this work, we found that it is not a sufficient condition because there are many more points of high MC density compared to traditionally given APs. This new finding was possible because we investigated the MC distribution throughout the whole skin section, whereas previous studies examined only some preselected points. These high MC density non-APs raise new subjects for acupuncture study: whether they are hitherto unknown novel APs or merely accidental coincidences. If the former is right, MCs could be used to find new APs. If the latter holds, it remains to find the common factors that gather many MCs in APs and non-APs. In addition, and more importantly, our method of studying the MC density throughout the whole skin has the advantage of providing quantitative information about the locations, depths, and sizes of the APs up to the submillimeter scale which was not given in previous studies [4–8]. This cytological method is a newly developed tool which is useful for characterizing the APS together with conventionally used anatomical and histological methods [20, 25, 26].

Our results are in agreement with the first quantitative analysis of the distribution of MCs in the skin of a mouse by Larsson and Sylven [18], despite the uses of different strains of mice (ICR versus Swiss albino) and different locations. We studied the ventral skin near the umbilicus, while they studied the interscapular regions of the dorsal skin. Both teams found a high density of MCs in the deep dermis and hypodermis, no MCs in the epidermis, and only very few in

the adventitia, skeletal muscles, and fascia. These results are also consistent with data for the human arm [19].

In our case, the average background number density of MCs in the deep dermis and hypodermis for abdominal skin was $0.3 \pm 0.1/(100\,\mu m)^2$. Larsson and Sylven reported that it was $2.4/(100\,\mu m)^2$ in the dermis and $0.74/(100\,\mu m)^2$ in the hypodermis [18]. Similarly, the MC density at nonacupoints near some APs of rats was reported to be 1.3~ $1.8/(100\,\mu m)^2$ [6]. In the case of human skin, Eady et al. reported $0.47/(100\,\mu m)^2$ [19], and Craig and Schwarz quoted $0.31/(100\,\mu m)^2$ in the dermis [27]. Large variations in the MC densities are known to exist, depending on the species, the individual, and the location in the skin. When we consider the different histological processes, different positions, and individual variations in the various studies, we can conclude that our number densities are in reasonable agreement with those published elsewhere. In addition, a mechanical calculation of the density of mast cells in dermal connective tissue may easily lead to different values because of the various deformations of the skin which can occur during the preparation of the sample [18, 19]. Therefore, the density should not be taken as a precise standard average value, which may not even be definable.

Nevertheless, regions having clumped high densities of MCs, which are the putative APs, were consistently observed and were in accord with previous results for rats [4, 5]. In this study, the average MC number densities were $3.3 \pm 1.7/(100\,\mu m)^2$, $2.7 \pm 0.9/(100\,\mu m)^2$, and $2.9 \pm 0.9/(100\,\mu m)^2$ at the CV 8 and the right and the left KI 16 APs, respectively, which are in agreement with the data for rats: $2.3 \pm 0.7/(100\,\mu m)^2$ at ST 36, $2.4 \pm 0.6/(100\,\mu m)^2$ at ST 31, and $2.3 \pm 0.6/(100\,\mu m)^2$ at ST 25 [6].

In the current work, we were able to infer the locations, depths, and sizes of the three APs, CV 8 and the left and the right KI 16 APs, by making use of the number density of MCs. The MCs were all in the dermis and the hypodermis and had oval shapes with sizes of about 200 μm, as shown in Table 2. The distance of the CV 8 AP from the umbilicus was $70.9 \pm 44.9\,\mu m$. The distances of the right and the left KI 16 APs from CV 8 were $541.8 \pm 184.8\,\mu m$ and $565.4 \pm 178.4\,\mu m$, respectively. These correspond to 0.4 ± 0.1 F-cun. We used the F-cun measurement of finger width [25]. In our case, 1 F-cun was approximately 1.3 mm. The F-cun measure of the KI 16 AP is 0.5 in traditional knowledge.

However, the regions highly populated with MCs did not always coincide with conventionally designated APs. For example, the next points from the KI 16 APs were only about 1,300 μm away from CV 8, as shown in Table 2. If they were ST 25 as in traditional Chinese medicine, the distance should be about 2,600 μm. The extra high MC density point at 1,300 μm might be a novel extra AP which does not exist in a human body. If this is true in general the MC density can be a novel cytological method to find extra APs. However, it might not be an AP at all. In this case, a subject to investigate in the future is the reason why some non-APs have high-density MCs. Currently the reason is not known either in traditional Chinese medicine or in Western medicine.

One obvious limitation of the current work is the failure to determine the boundaries enclosing the APs by using the MC distribution. The reason was the limitation of the staining dye toluidine-blue that shows MCs well but cannot show many other components of the skin tissue. At the present time, the dye to stain the boundary cells or tissues of the APs is not known. For example, Hemacolor technique stained many other components of the primo node but it did not specifically show the boundary tissue [22]. So, it remains an important task to find the right dye to stain the boundary tissue of the APs, which we hope to solve in the future.

Interestingly, we found that rats and mice are different with respect to the fat tissue underneath the fascia. The fat pad of a mouse was a round or oval disk shape of about 1,000 μm in diameter (Figure 3(a)). A rat has a long fat band, as compared to the fat pad of a mouse. Both the long fat band in a rat and the fat pad in a mouse are populated with MCs. The fat band of a rat has a PVS in it, and high densities of MCs in the PVS have recently been found in several works on the primo nodes in the abdominal cavity [9, 21–23].

The methods used in this study can be used to investigate the distributions of MCs for other skin areas to determine the gross anatomical features of APs. Similar studies on pathological conditions in mice will be useful for investigating the roles of MCs in the treatment of diseases. One medically significant application of nanotechnology to detect and monitor ST 36 by measuring the serotonin secreted by the MCs residing in the APs was proposed by Li et al. [24]. For this purpose, knowledge of both the locations and the depths of the APs, for which the current work provides useful information, is essential.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to the content of this manuscript.

Acknowledgments

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References

[1] Y.-S. Son, H.-J. Park, O.-B. Kwon, S.-C. Jung, H.-C. Shin, and S. Lim, "Antipyretic effects of acupuncture on the lipopolysaccharide-induced fever and expression of interleukin-6 and interleukin-1β mRNAs in the hypothalamus of rats," *Neuroscience Letters*, vol. 319, no. 1, pp. 45–48, 2002.

[2] J. Kalesnikoff and S. J. Galli, "Antiinflammatory and immunosuppressive functions of mast cells," in *Suppression and Regulation of Immune Responses*, vol. 677 of *Methods in Molecular Biology*, pp. 207–220, Springer, 2011.

[3] T. C. Theoharides, P. Valent, and C. Akin, "Mast cells, mastocytosis, and related disorders," *The New England Journal of Medicine*, vol. 373, pp. 163–172, 2015.

[4] Z. Zhu and R. Xu, "Morphometric observation on the mast cells under the acupuncture meridian lines," *Zhen Ci Yan Jiu*, vol. 15, pp. 157–158, 1990 (Chinese).

[5] A. M. Zhong, J. L. Wu, and Y. L. Hu, "Study of correlation between the mast cell and acupoint," *Shijie Zhenjiu Zazhi*, vol. 4, pp. 53–58, 1994 (Chinese).

[6] Y. Deng, Z. Fu, H. Dong, Q. Wu, and X. Guan, "Effects of electroacupuncture on the subcutaneous mast cells of zusanli acupoint in rat with unilateral sciatic nerve transection," *Zhen Ci Yan Jiu*, vol. 21, no. 3, pp. 46–49, 1996 (Chinese).

[7] Y. Deng, T. Zeng, Y. Zhou, and X. Guan, "The influence of electroacupuncture on the mast cells in the acupoints in stomach meridian," *Zhen Ci Yan Jiu*, vol. 21, pp. 68–70, 1996 (Chinese).

[8] D. Zhang, G. H. Ding, X. Y. Shen et al., "Role of mast cells in acupuncture effect: a pilot study," *Explore*, vol. 4, no. 3, pp. 170–177, 2008.

[9] H. Jang, J. Yoon, H. Gil et al., "Observation of a flowing duct in the abdominal wall by using nanoparticles," *PLoS ONE*, vol. 11, no. 3, Article ID e0150423, 2016.

[10] B. H. Kim, "The Kyungrak system," *J Jo Sun Med*, vol. 108, pp. 1–38, 1965.

[11] D. E. Smith, "The tissue mast cell," *International Review of Cytology*, vol. 14, pp. 327–386, 1963.

[12] R. A. Lewis and K. F. Austen, "Nonrespiratory functions of pulmonary cells: the mast cell," *Federation Proceedings*, vol. 36, no. 13, pp. 2676–2683, 1977.

[13] M. Maurer, T. Theoharides, R. D. Granstein et al., "What is the physiological function of mast cells?" *Experimental Dermatology*, vol. 12, no. 6, pp. 886–886, 2003.

[14] C. Noli and A. Miolo, "The mast cell in wound healing," *Veterinary Dermatology*, vol. 12, no. 6, pp. 303–313, 2001.

[15] C. Johnson, V. Huynh, L. Hargrove et al., "Inhibition of mast cell-derived histamine decreases human cholangiocarcinoma growth and differentiation *via* c-Kit/stem cell factor-dependent signaling," *The American Journal of Pathology*, vol. 186, no. 1, pp. 123–133, 2016.

[16] P. C. W. Fung, "Probing the mystery of Chinese medicine meridian channels with special emphasis on the connective tissue interstitial fluid system, mechanotransduction, cells durotaxis and mast cell degranulation," *Chinese Medicine*, vol. 4, article 10, 6 pages, 2009.

[17] D. D. Metcalfe, D. Baram, and Y. A. Mekori, "Mast cells," *Physiological Reviews*, vol. 77, no. 4, pp. 1033–1079, 1997.

[18] L.-G. Larsson and B. Sylven, "The mast cell reaction of mouse skin to some organic chemicals I. Estimation of the relative number of mast cells in normal mouse skin," *Cancer Research*, vol. 7, no. 11, pp. 676–679, 1947.

[19] R. A. Eady, J. T. Cowen, T. F. Marshall, V. Plummer, and M. W. Greaves, "Mast cell population density, blood vessel density and histamine content of normal human skin," *British Journal of Dermatology*, vol. 100, pp. 623–633, 1979.

[20] L. Mingfu, D. Xiaotong, S. Xiaojing, J. Jin, Z. Jinling, and H. Ying, "Study on the dynamic compound structure composed of mast cells, blood vessels, and nerves in rat acupoint," *Evidence-based Complementary and Alternative Medicine*, vol. 2013, Article ID 160651, 4 pages, 2013.

[21] B. S. Kwon, C. M. Ha, S. Yu, B.-C. Lee, J. Y. Ro, and S. Hwang, "Microscopic nodes and ducts inside lymphatics and on the surface of internal organs are rich in granulocytes and secretory granules," *Cytokine*, vol. 60, no. 2, pp. 587–592, 2012.

[22] C. J. Lim, S. Y. Lee, and P. D. Ryu, "Identification of primo-vascular system in abdominal subcutaneous tissue layer of rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 751937, 13 pages, 2015.

[23] H. Gil, K.-H. Bae, L. Kim, S. Kim, and K.-S. Soh, "Number density of mast cells in the primo nodes of rats," *JAMS Journal of Acupuncture and Meridian Studies*, vol. 8, no. 6, pp. 288–293, 2015.

[24] Y. Li, L. Tang, Y. Ning et al., "In vivo monitoring of serotonin by nanomaterial functionalized acupuncture needle," *Scientific Reports*, vol. 6, no. 1, Article ID 28018, 2016.

[25] World Health Organization, *WHO Standard Acupuncture Point Locations in the Western Pacific Region*, World Health Organization, Geneva, Switzerland, 2008.

[26] H. M. Langevin and J. A. Yandow, "Relationship of acupuncture points and meridians to connective tissue planes," *The Anatomical Record*, vol. 269, no. 6, pp. 257–265, 2002.

[27] S. S. Craig and L. B. Schwartz, "Tryptase and chymase, markers of distinct types of human mast cells," *Immunologic Research*, vol. 8, no. 2, pp. 130–148, 1989.

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Research Article

Laser Acupuncture Exerts Neuroprotective Effects via Regulation of *Creb*, *Bdnf*, *Bcl-2*, and *Bax* Gene Expressions in the Hippocampus

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Acupuncture has a positive effect on cognitive deficits. However, the effects of laser acupuncture (LA) on cognitive function and its mechanisms of action are unclear. The present study aimed to evaluate the effects of LA on middle cerebral artery occlusion-(MCAO-) induced cognitive impairment and its mechanisms of action. Transient focal cerebral ischemia was modeled in adult Sprague-Dawley rats by MCAO. After LA or manual-acupuncture (MA) treatment at the GV20 and HT7 for 2 weeks, hippocampal-dependent memory was evaluated using the Morris water maze (MWM) test. The hippocampus was dissected to analyze choline acetyltransferase (ChAT) immunoreactivity and *Creb*, *Bdnf*, *Bcl-2*, and *Bax* gene expressions. MWM test demonstrated a significant improvement in hippocampal-dependent memory in the MCAO rats after LA treatment. LA treatment significantly reversed the postischemic decrease in ChAT immunoreactivity in the hippocampal CA1 region. LA treatment significantly normalized gene expression in the hippocampus which had been altered by MCAO, especially upregulating gene expression of Creb, Bdnf, and Bcl-2 and downregulating gene expression of Bax. This study suggests that LA treatment could improve cognitive impairment in MCAO rats to enhance the cholinergic system in the hippocampal CA1 region and to exert a neuroprotective effect by regulating *Creb*, *Bdnf*, *Bcl-2*, and *Bax* gene expressions.

1. Introduction

In Asian countries, such as China, Korea, Japan, and Vietnam, acupuncture has been one of the most widely used treatment methods in traditional medicine for thousands of years [1]. In complementary and alternative medicine, it is frequently utilized for neurological disorders, such as stroke and dementia, because of its neuroprotective effects and its ability to improve poor cognitive function [2, 3]. In addition to conventional manual acupuncture (MA), which only uses needles, other acupuncture approaches, such as electrostimulation, have been reported [4, 5]. One of the alternative techniques in acupuncture is laser acupuncture (LA), which utilizes laser irradiation. LA treatment involves stimulation of the appropriate acupuncture points based on acupuncture theory, using a low-intensity, nonthermal laser [6]. Several studies have reported that LA treatment alleviates cognitive deficits in animal models through various mechanisms, such as reducing oxidative stress and protecting against damage to cholinergic and dopaminergic neurons [7–9].

Cerebrovascular disease is the second most common cause of acquired cognitive impairment and dementia and has been reported to contribute to impaired cognition in neurodegenerative dementia [10]. The hippocampus is one of the most important regions of the brain with respect to cognition, learning, and memory, and the hippocampal CA1 region has

been shown to be particularly vulnerable to ischemic insult [11]. In previous studies, after induction of focal cerebral ischemia by middle cerebral artery occlusion (MCAO), neurodegeneration was reported in the hippocampal CA1 region, accompanied by long-term cognitive deficits [12, 13]. Neuron survival after such ischemic brain injury has been shown to be mediated by regulation of cAMP response element- (CRE-) mediated gene expressions, including cAMP response binding protein *(Creb)* [14], brain-derived neurotrophic factor *(Bdnf)* [15], and B-cell lymphoma 2 *(Bcl-2)* [16] expressions.

In our previous study, when LA treatment was applied to two acupuncture points (HT9 and LR1) in MCAO rats, an antiapoptotic effect was observed, with counterregulation of *Bcl-2* and Bcl-2-associated X protein *(Bax)* gene expressions [17]. Several previous studies have reported that acupuncture stimulation at the acupuncture points GV20 [18] and HT7 [19] in animal models showed potential neuroprotective effects and improvement of cognitive deficits. However, the molecular mechanisms associated with LA's neuroprotective effects and alleviation of cognitive deficits after brain ischemia are unclear. Therefore, the present study aimed to evaluate the effects of LA at the acupuncture points GV20 and HT7 on MCAO-induced cognitive impairment and its mechanisms of action.

2. Materials and Methods

2.1. Animals and the MCAO Model. The study included 8-week-old male Sprague-Dawley (SD) rats weighing 260–300 g. The rats were acclimated in a temperature-controlled (22 ± 3°C) environment with a constant 12-hour light/dark cycle and ad libitum access to food and water 3 days prior to surgery. All procedures in this experiment were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications number 8023), revised in 1996, and were approved by the Institutional Animal Care and Use Committee of Dongshin University (2015-04-04). A rat model of transient focal cerebral ischemia was created by occlusion of the left middle cerebral artery, in accordance with the surgical procedure presented by Longa et al. [20]. In brief, the rats were subjected to inhalation anesthesia (following induction with 5% isoflurane, anesthesia was maintained at a concentration of 2%). An incision was made in the neck at the midline, and the left common carotid artery was exposed between the sternocleidomastoid and omohyoid muscles. The terminal branch of the left internal carotid artery (1 cm from where it branches from the left common carotid artery) was perforated with microvascular scissors, and an intraluminal filament (0.28 × 20 mm, rounded tip) covered in dental impression material (Durelon, ESPE, Seefeld, Germany) was inserted.

2.2. Experimental Design. In order to observe the effects of LA on cognitive impairment in the MCAO rat model, we arbitrarily divided 24 SD rats into the following four groups: the naïve, control (MCAO only), MA (MCAO + MA), and LA (MCAO + LA) groups.

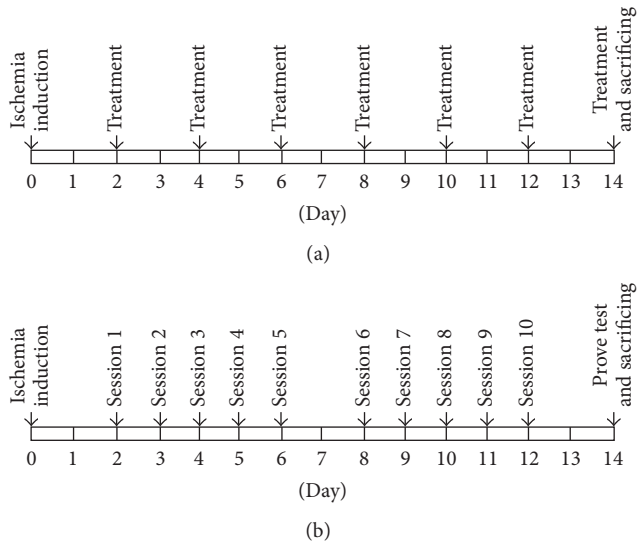

Figure 1: Schematic design of the experimental procedures for acupuncture treatment (a) and the Morris water maze test (b).

2.3. Acupuncture Treatment. After 48 postoperative hours, acupuncture treatment was performed 4 hours before the Morris water maze task in the behavioral test period. Acupuncture treatment was performed using either an invasive LA apparatus (Ellise-005, Ver. 1.0.1, Wontech, Daejeon, Korea; for the LA group; Table 1) or acupuncture needles (0.20 × 30 mm, sterilized disposable stainless steel, HLMedical, Yeoju, Korea; for the MA group). The acupuncture points Baekhoe (GV20) and Sinmun (HT7) were stimulated for 5 minutes, once every 2 days, for 2 weeks, resulting in a total of seven stimulations at each point (Figure 1(a)). HT7 was stimulated unilaterally on the right side (i.e., contralateral to the left MCAO). GV20 was anatomically located at the intersection of the line connecting the apexes of the two auricles and the median line of the head, while HT7 was located at the transverse crease of the wrist of the forepaw, radial to the tendon of the flexor carpi ulnaris muscle [21]. The LA stimulation parameters were as follows: laser-guided needles (200 μmin diameter, with an optic fiber 125 μm in diameter); diode laser irradiation; wavelength, 650 nm; intensity, 30 mW; and repetition rate, 100 Hz. For both LA and MA, the needles were inserted at GV20 (obliquely) and HT7 (perpendicularly) to a depth of approximately 2-3 mm. The naïve and control groups only underwent 5 minutes of inhalation anesthesia. A rat undergoing LA treatment is presented in Figure 2.

2.4. Morris Water Maze Test. Environmental factors relevant to the Morris water maze task were based on the protocol presented by Vorhees and Williams [22]. In brief, the water maze consisted of a polypropylene circular pool (diameter, 120 cm; height, 50 cm), and the pool was divided into four identical quadrants (northeast, northwest, southeast, and southwest). A water temperature of 22 ± 1°C was maintained inside the pool, and ink was added to make the water opaque. In the Morris water maze task, a platform (diameter, 20 cm;

Table 1: Specifications of the laser acupuncture system.

Property	Specification
Irradiation type	Diode laser
Wavelength	532 nm, 650 nm, 830 nm, 905 nm, 1064 nm
Operating current	Each wavelength up to 30 mW
Core/cladding diameter	50/125 μm
Pulse duration	1 min; max, 99 min
Repetition rate	1 Hz; max, 200 Hz

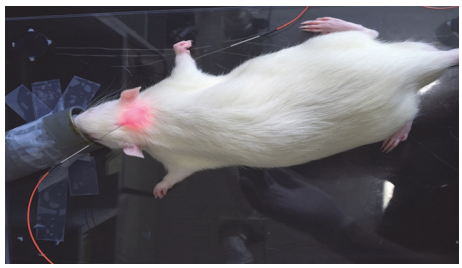

Figure 2: An image of laser acupuncture treatment being applied to the two acupuncture points GV20 and HT7.

height, 32 cm; and depth, 1-2 cm below the surface of the water) was located in the center of one of the four quadrants. The pool was surrounded by numerous external clues. A camera lens (CS Mount 1/3″ 4 mm Fixed Focus Manual Iris Lens/T0412FICS, CBC AMERICAS, Cary, NC) was installed and fixed above the pool, and automated video tracking of the swim paths of the rats was implemented in all trials, using a tracking program (Smart ver. 3.0.01, Panlab/Harvard Apparatus, Holliston, MA) linked to the camera.

2.4.1. Acquisition Trial. A hidden platform located in the northwest quadrant was the only place for the rats to avoid the water. A rat was placed arbitrarily in one of the other quadrants facing the wall of the pool. When the rat reached the platform, the timer was stopped immediately. If a rat was unable to find the platform within 60 seconds, it was guided gently to the platform, and the escape latency time was recorded as 60 seconds. When a rat climbed onto the platform, it was not removed immediately, but rather a 15-second intertrial interval was provided for the rat to remember the area around the pool. The rats were tested in sessions consisting of four trials a day, and over a 2-week period, a total of 10 sessions were performed for each rat (Figure 1(b)). The escape latency time and swim speed were measured.

2.4.2. Probe Trial. The probe trial was performed 24 hours after the final acquisition trial (Figure 1(b)). During the probe trial, the platform was removed, and the rats were allowed to swim freely for 120 seconds. The distance to the target quadrant and the percentage of time spent in the target quadrant were measured.

2.5. Immunohistochemistry. Immunohistochemical analysis was performed to detect choline acetyltransferase (ChAT) activity. The rats were placed under deep anesthesia using 25% urethane (Sigma, St. Louis, MO) and were perfused through the heart with 200 mL of normal saline (0.9%), followed by 300 mL of 4% formalin (per rat) in 0.1 M phosphate-buffered saline (PBS). The brain was removed, and following 2 hours of postfixation, it was cryoprotected overnight at 4°C using 30% sucrose in 0.1 M PBS. A cryostat (Cool Ace series CA-1500, Eyela, Tokyo, Japan) was used to produce 30 μm thick coronal sections of the hippocampus. The hippocampal slices were washed several times with 0.1 M PBS, and they were then placed on glass slides, dried at 37°C, and stored in a refrigerator. Primary sheep ChAT antibody (1 : 500, monoclonal, Millipore, Billerica, MA) was used to immunostain the slices for ChAT expression. The primary antibody was prepared by diluting the original solution 500-fold in 0.1 M PBS with 0.1% sodium azide (Sigma) buffer. The slices were soaked in the primary antibody at 4°C for 24 hours. They were then washed at least thrice with 0.1 M PBS and were treated with biotinylated universal secondary antibody (Quick Kit; Vector Laboratories, Burlingame, CA) at 37°C for 30 minutes. The slices were again washed at least thrice with 0.1 M PBS

Table 2: PCR primer sequences.

Gene	Primer sequence (forward and reverse)	Product size (base pair)	Annealing temperature (°C)
Gapdh	5′-TGCATCCTGCACCACCAACT-3′ 5′-CGCCTGCTTCACCACCTTG-3′	349	56
Creb	5′-TACCCAGGGAGGAGCAATAC-3′ 5′-GAGGCAGCTTGAACAACAAC-3′	183	51
Bdnf	5′-CAGGGGCATAGACAAAAG-3′ 5′-CTTCCCCTTTTAATGGTC-3′	153	57
Bcl-2	5′-TTGTGGCCTTCTTTGAGTTCGGTG-3′ 5′-GGTGCCGGTTCAGGTACTCAGTCA-3′	168	55
Box	5′-CCTGTGCACCAAGGTGCCGGAACT-3′ 5′-CCACCCTGGTCTTGGATCCAGCCC-3′	498	55

and were then soaked in streptavidin peroxidase preformed complex (Quick Kit; Vector Laboratories) at 37°C for 30 minutes. The slices were again washed at least thrice in 0.1 M PBS and were then incubated with diaminobenzidine (DAB; Sigma) for 1 min. Finally, the tissues were washed in 0.1 M PBS and briefly rinsed in distilled water. After dehydrating the tissues, the stained tissues were observed at 40x magnification using a light microscope (Eclipse 80i, Nikon, Tokyo, Japan). The ChAT density in the hippocampus was measured using the Scion image program (Scion Corp., Frederick, MD).

2.6. Total RNA Isolation and RT-PCR. For RNA isolation, the hippocampus was dissected out from each group. After decapitation, the brain was removed as quickly as possible and stored at −80°C until use. The tissue of the left hippocampus was homogenized in 800 μL TRIZOL reagent (Roche Diagnostics GmbH, Mannheim, Germany). Then, 200 μL of chloroform (Sigma) was added and mixed well by shaking for 15 seconds. The mixture was allowed to rest for 15 minutes at 24 ± 1°C. The mixture was then centrifuged at 14,000 rpm for 15 minutes at 4°C. The supernatant was collected; 500 μL of isopropanol (Sigma) was added to the supernatant, and the mixture was allowed to rest for 5 minutes at 24 ± 1°C. This mixture was then centrifuged at 14,000 rpm for 8 minutes at 4°C, and the RNA pellet was collected. The RNA pellet was mixed with refrigerated 70% ethanol and DEPC. This mixture was then centrifuged at 7,500 rpm for 5 minutes at 4°C, and the liquid was removed, leaving only the pellet. The remaining ethanol was dried for 5 minutes at 24 ± 1°C. The pellet was dissolving in DEPC-treated water, and the optical density was measured using a spectrophotometer (Biophotometer, Eppendorf, Hamburg, Germany) in order to determine the purity and concentration of RNA. cDNA was synthesized using the total RNA with reverse transcriptase (Bioneer, Daejeon, Korea). The mRNA expression levels of *Creb, Bdnf, Bcl-2, and Bax* were determined using reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR was performed using a Mastercycler Gradient (Eppendorf) with the following conditions: for glyceraldehyde-3-phosphate dehydrogenase *(Gapdh)*, 28 cycles of denaturation at 95°C for 40 s, annealing at 56°C for 40 s, and extension at 72°C for 90 s; for *Creb*, 27 cycles of denaturation at 95°C for 30 s, annealing at 51°C for 30 s, and extension at 72°C for 30 s; for *Bdnf*, 27 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 30 s; for *Bcl-2*, 35 cycles of denaturation at 95°C for 40 s, annealing at 55°C for 40 s, and extension at 72°C for 90 s; and for *Bax*, 35 cycles of denaturation at 95°C for 40 s, annealing at 55°C for 40 s, and extension at 72°C for 90 s. The sequences used in RT-PCR are shown in Table 2. The PCR products were subjected to electrophoresis at 100 V using 0.5x TBE buffer (80 mM Tris-HCL, 80 mM boric acid, 2 mM EDTA, and pH 8.3) in a 1.5% agarose gel containing ethidium bromide (EtBr, 10 mg/mL). After electrophoresis, the gel was imaged using an Image Station (Kodak, Rochester, NY), and the density of each band was analyzed using Alphaease FC StandAlone Software (Alpha Innotech, San Leandro, CA). *Creb, Bdnf, Bcl-2*, and *Bax* mRNA expressions were normalized relative to the *Gapdh* mRNA expression.

2.7. Statistical Analysis. All data are presented as means ± standard errors of means (SEMs). The Morris water maze data were analyzed with repeated measures analysis of variance (ANOVA). Statistical significance between the groups was verified using Tukey's post hoc test. The immunohistochemistry and PCR data were analyzed using one-way ANOVA followed by Tukey's post hoc test. All statistical analyses were performed using SPSS Software (version 21.0; IBM Corp., Armonk, NY). A p value < 0.05 was considered statistically significant.

3. Results

3.1. Effects of LA on MCAO-Induced Learning and Memory Deficits. The representative swim paths in the Morris water maze test are presented in Figure 3. In the acquisition trials, all groups showed a gradual decrease in the escape latency to reach the hidden platform as the number of sessions increased (Figure 4(a)). The escape latency time was significantly lower in the naïve group than in the control group ($p < 0.01$ for sessions 3–5 and 7; $p < 0.001$ for sessions 6 and 8–10). The delayed escape latency time in the LA group showed significant improvement when compared with the

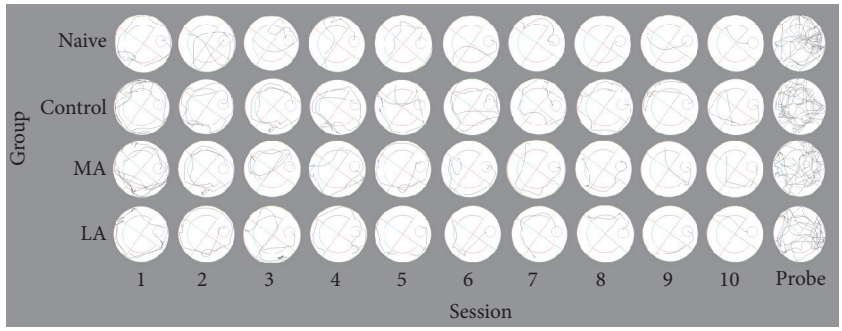

Figure 3: Representative swim paths in the Morris water maze test. The swim paths were analyzed using automated video tracking for all acquisition and probe trials.

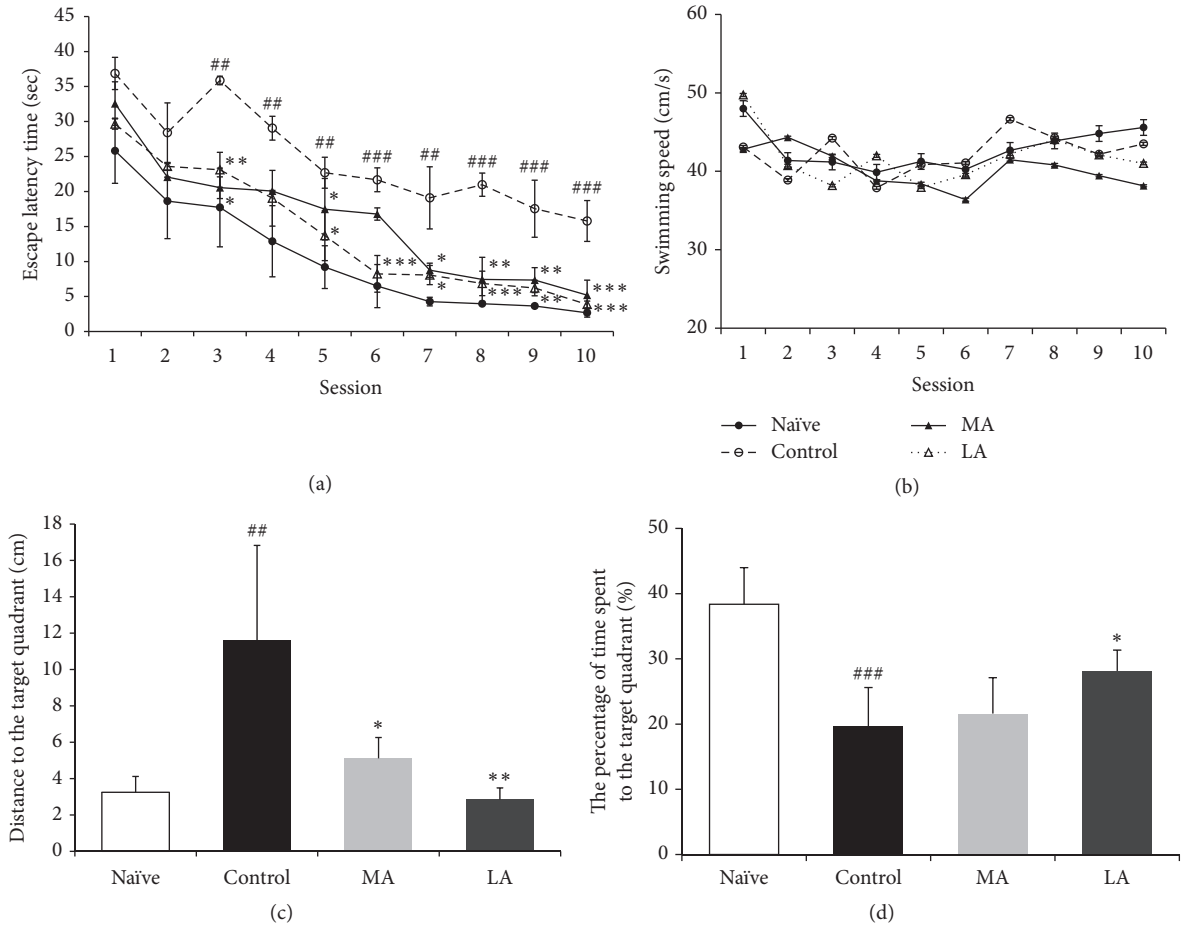

Figure 4: Results of the Morris water maze test. (a) Escape latency time and (b) swim speed during the acquisition trials with a hidden platform. (c) Distance to the target quadrant and (d) percentage of time spent in the target quadrant during the probe trial. Data were analyzed using two-way ANOVA, followed by Tukey's post hoc test. Vertical bars indicate the SEM. Data are expressed as mean ± SEM ($n = 6$ in each group). $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with the naïve group; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the control group.

delayed escape latency time in the control group ($p < 0.05$ for sessions 3, 5, and 7; $p < 0.01$ for session 9; and $p < 0.001$ for sessions 6, 8, and 10). Additionally, the delayed escape latency time in the MA group showed significant improvement when compared with the delayed escape latency time in the control group ($p < 0.05$ for sessions 6 and 7; $p < 0.01$ for sessions 3, 8, and 9; and $p < 0.001$ for session 10). The delayed escape latency time was not significantly different between the LA and MA groups ($p = 0.128$). Additionally, there was no between-group difference in the swim speed ($p = 0.147$, Figure 4(b)). In the probe trial, the distance to the target quadrant ($F(3,20) = 8.806$, $p < 0.01$; Figure 4(c)) and the

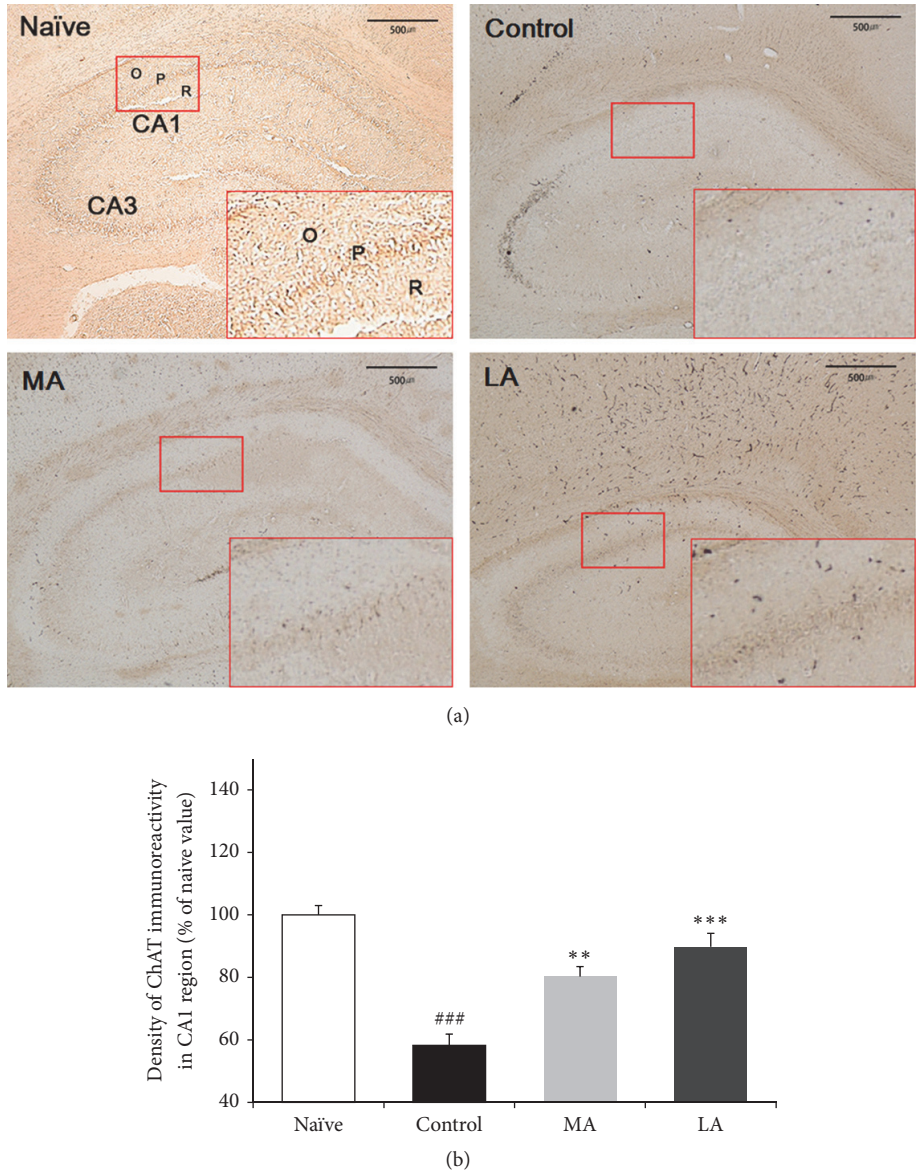

Figure 5: Results of choline acetyltransferase (ChAT) analysis. (a) Representative images showing the density of ChAT in the hippocampus. Red box: CA1 region; O: stratum oriens; P: stratum pyramidale; and R: stratum radiatum. The scale bar represents 500 μm. (b) Percentage (±SE) values for ChAT immunoreactivity in the hippocampal CA1 region after the Morris water maze task. Immunohistochemical data were analyzed using one-way ANOVA, followed by Tukey's post hoc test ($n = 3$ in each group). Vertical bars indicate the SEM. $^{###}p < 0.001$ compared with the naïve group; $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with the control group.

time spent in the target quadrant ($F(3,20) = 13.355$, $p < 0.001$; Figure 4(d)) showed significant between-group differences. The distance to the target quadrant was significantly higher and the time spent in the target quadrant was significantly lower in the control group than in the naïve group ($p < 0.01$ and $p < 0.001$, resp.). The distance to the target quadrant was significantly lower in the LA and MA groups than in the control group (MA group: $p < 0.05$; LA group: $p < 0.01$; Figure 4(c)). However, the time spent in the target quadrant was higher in only the LA group than in the control group ($p < 0.05$, Figure 4(d)).

3.2. Effects of LA on MCAO-Induced ChAT Immunoreactivity Reduction. After the behavior test, immunohistochemical analysis was performed to verify cholinergic neuronal cell loss in the rat brains with induced MCAO (Figure 5(a)). The results of ChAT analysis showed that the ChAT density in the hippocampal CA1 region was significantly lower in the control group than in the naïve group (40.23 ± 3.40 (62.85 ± 5.29%) versus 64.29 ± 1.92 (100.0 ± 2.99%), $p < 0.001$; Figure 5(b)). When the ChAT density was compared using one-way ANOVA, we noted significant between-group differences ($F(3,8) = 44.652$, $p < 0.001$). LA and MA treatment significantly reversed the MCAO-induced decrease in

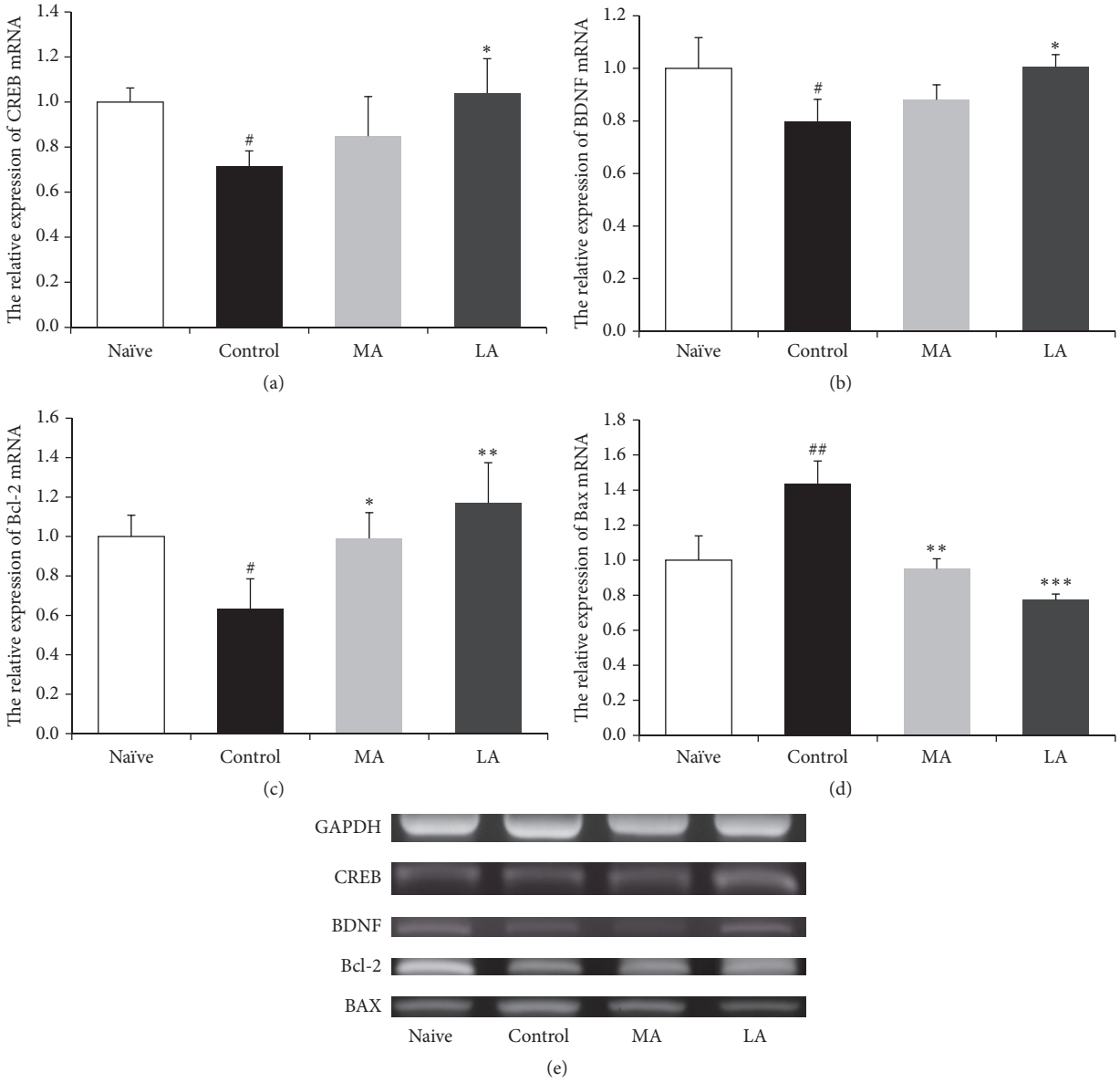

Figure 6: Gene expressions in the hippocampal CA1 region. RT-PCR is used to measure the fold change in the gene expressions of (a) cAMP response element-binding protein (CREB), (b) brain-derived neurotrophic factor (BDNF), (c) B-cell lymphoma 2 (Bcl-2), and (d) Bcl-2-associated X protein (Bax). The mRNA levels are normalized relative to the Gapdh mRNA level. RT-PCR data were analyzed using one-way ANOVA, followed by Tukey's post hoc test ($n = 3$ in each group). Vertical bars indicate SEM. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the naïve group; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the control group.

ChAT density in the hippocampal CA1 region (57.72 ± 2.77 (89.79 ± 4.31%), $p < 0.001$ and 50.53 ± 3.89 (78.59 ± 6.05%), $p < 0.01$, resp.).

3.3. Effects of LA on MCAO-Induced CREB and CRE-Mediated Gene Expressions. RT-PCR was performed to investigate the effects of LA on the mRNA expressions of *Creb, Bdnf, Bcl-2*, and *Bax* in the hippocampal CA1 region of MCAO rats (Figure 6). The mRNA expressions of *Creb* ($F(3,8) = 5.651$, $p < 0.05$; Figure 6(a)), *Bdnf* ($F(3,8) = 6.165$, $p < 0.05$; Figure 6(b)), *Bcl-2* ($F(3,8) = 8.632$, $p < 0.05$; Figure 6(c)), and *Bax* ($F(3,8) = 31.011$, $p < 0.001$; Figure 6(d)) showed significant between-group differences. The *Creb*, *Bdnf*, and *Bcl-2* gene expressions showed significant downregulation by approximately 0.7-, 0.8-, and 0.6-fold and the expression of *Bax* showed a significant upregulation by 1.4-fold in the control group when compared with the corresponding expressions in the naïve group ($p < 0.05$, $p < 0.05$, $p < 0.05$, and $p < 0.01$, resp.). The *Creb*, *Bdnf*, and *Bcl-2* gene expressions were significantly higher and the expression of *Bax* was significantly lower in the LA group than in the control group ($p < 0.05$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, resp.; Figure 6). Conversely, the *Bcl-2* gene expression was significantly higher and the expression of *Bax*

was significantly lower in the MA group than in the control group ($p < 0.05$ and $p < 0.01$, resp.).

4. Discussion

Acupuncture is a simple, flexible method in traditional medicine, with few adverse effects [23]. It has recently received attention for its ability to alleviate cognitive deficits and its neuroprotective effects after brain ischemia [2, 3]. LA is a new acupuncture technique using the concept of low-level laser therapy (LLLT), which stimulates acupuncture points based on traditional meridian theory [6, 24]. Because LLLT uses a low irradiation level laser and there is no risk of excessive heat, its mechanism is believed to be associated with a photochemical effect, rather than a thermal effect [6]. Previous studies have reported that LA improves cognitive deficits and memory impairment in various neurological disorders, such as depression [25], autism [9], and Parkinson's disease [8].

In the present study, invasive LA was used to stimulate the acupuncture points GV20 and HT7 for 5 minutes at a wavelength of 650 nm and a power of 30 mW. In conventional noninvasive LA, the acupuncture points are only irradiated with a light source, and, therefore, the procedure has the advantage of being needle- and pain-free [6, 24]. However, it is difficult to make an objective evaluation about the efficacy of LA itself, as laser energy transmission is restricted by the structural characteristics of the skin [26, 27]. Furthermore, noninvasive, low-intensity LA irradiation cannot produce activation of mechanical signal transduction pathways, which results from reorganization of the collagen by acupuncture needles, and it has not been clearly demonstrated whether the photon-mediated effects of LA itself have the same signal transduction pathways as the needle-mediated mechanical effects [28, 29]. In previous studies, stimulation of the acupuncture points HT9 and LR1 with invasive LA at 658 nm in MCAO rats produced antiapoptotic and neuroprotective effects that were similar to the effects with MA, while the efficacy of LA was found to be superior [17]. In LLLT, red and infrared wavelengths of 600–1300 nm and a power of 1–100 mW are the usual stimulation parameters applied for laser acupuncture because there is little absorption by tissues, penetration is excellent, and there is no major damage to tissues with these parameters [26, 30, 31]. Previous studies have reported on the safety of repeated LA treatments in rats (wavelength, 650 nm; maximum power, 60 mW; 5 minutes of stimulation once every 2 days for 16 days) [32].

In the traditional acupuncture theory, the points GV20 and HT7 have been used for a long time in neurological and psychiatric disorders, such as insomnia, epilepsy, and amnesia [33]. A meta-analysis concluded that acupuncture stimulation at the point GV20 reduced the area of infarction in animal models of experimental ischemic stroke, improved neurological function scores, and showed a potential neuroprotective effect [18]. A recent study reported that acupuncture stimulation at the point HT7 in a rat model with cognitive deficit reduced damage to cholinergic neurons and showed a neuroprotective effect by regulating *Creb* and *Bdnf* gene expressions [19].

The Morris water maze is a behavioral instrument used to evaluate cognitive function by measuring spatial learning and memory in rats, based on a logical experimental design [22]. After the spatial working memory information obtained during acquisition trials has been encoded, the spatial reference memory information is retrieved in the subsequent probe trial [34]. The performances in both types of trials were significantly lower in the MCAO rats than in the naïve rats (Figures 4(a), 4(c), and 4(d)); however, there was no significant between-group difference in the mean swim speed (Figure 4(b)). This indicates that MCAO did not cause a motor function deficit in the rat model [35] but rather caused spatial learning and memory impairments due to cognitive function deficits [36]. The reduced working memory and reference memory were both significantly normalized with LA treatment (Figures 4(a), 4(c), and 4(d)). These results indicate that LA can improve impaired hippocampal-dependent learning and memory in MCAO rats.

Brain regions that are supplied by the middle cerebral artery, such as the parietal cortex, hippocampus, and striatum, show severe neural injury after occlusion-induced cerebral ischemia [20]. The hippocampal CA1 region, which is involved in learning, memory, and cognitive function, is especially vulnerable to ischemic insult [11]. Cholinergic neurons originating in the medial septum project to the cortex and hippocampus, and this plays an important role in acetylcholine-related cognitive function [37]. Degeneration of cholinergic innervation is one of the causes of memory decay [38], and a previous study reported a cholinergic deficit in vascular dementia patients [39]. ChAT is involved in the synthesis, storage, and release of acetylcholine and is therefore used as a marker for cholinergic neurons in the hippocampal CA1 region [37]. In the present study, MCAO reduced ChAT immunoreactivity in the CA1 region (Figure 5), indicating that the cholinergic input from the medial septum to the hippocampus was damaged after ischemia [40]. LA significantly restored the decreased ChAT density in the CA1 region (Figure 5), suggesting that it has a beneficial effect on the cognition-associated cholinergic system.

We confirmed that LA treatment in the MCAO-induced cerebral ischemia model rat improved spatial learning and memory and restored ChAT activity in the CA1 region. It has been previously reported that after cerebral ischemia, CREB acts as an important contributor to the survival of neurons by increasing the expression of CRE-mediated genes, including *Bdnf* and *Bcl-2* [14–16]. In the present study, MCAO downregulated the expressions of *Creb* and *Bdnf* in the hippocampal CA1 region in the model rats; however, the expressions were significantly normalized with LA stimulation (Figures 6(a) and 6(b)). In a previous study, increased CREB activity reduced neuronal cell loss in the hippocampal CA1 region, which is vulnerable to ischemic insult, suggesting that ischemic brain injury has an effect on the CRE-mediated transcription system [41]. BDNF is a downstream neuroprotective target of CREB that is involved in neuronal survival after ischemia [14], and it is a neurotrophic factor that can phosphorylate CREB [42]. Previous studies have indicated that the positive-feedback loop between CREB and BDNF could become active in several

neuronal populations in response to ischemic brain injury [14, 42]. Additionally, BDNF has been reported to inhibit caspase-3 activity and increase the expression of the *Bcl-2* gene, preventing apoptotic cell death, and thereby reducing ischemic brain injury [15].

The antiapoptotic protein Bcl-2 is another CRE-mediated protein that contributes to neuronal survival [14]. Programmed cell death (PCD) is a factor in delayed neuronal death that occurs after ischemia; however, such apoptotic processes are regulated by the expression ratio of the *Bcl-2* gene and the proapoptotic *Bax* gene [43, 44]. In our study, MCAO reduced *Bcl-2* gene expression and increased *Bax* gene expression in the CA1 region of the rat model (Figures 6(c) and 6(d)). This Bcl-2 dysfunction has been reported to exacerbate ischemic neuronal injury [45]. LA stimulation significantly normalized this imbalance, suggesting that its antiapoptotic effect in the hippocampal CA1 region was the result of significant counterregulation of *Bcl-2* and *Bax* gene expressions [17]. Our results indicate that LA can induce upregulation of *Creb* and CRE-mediated gene expressions and exert a neuroprotective effect in the hippocampal CA1 region.

In the present study, we determined the effects of LA on cognitive impairment in MCAO rats and compared the effects with those of MA. The mechanisms associated with the effectiveness of LA in the treatment of cognitive improvement are unclear. On the other hand, MA has been reported to reduce cognitive decline by stimulating cellular signaling via the needle-mediated mechanical effect, regulating apoptosis, reducing oxidative stress, stabilizing energy metabolism, and restoring synaptic transmission [46]. Our study found that MA stimulation at the points GV20 and HT7 in MCAO rats significantly improved learning and memory deficits in the behavioral test, significantly restored ChAT activity in the hippocampal CA1 region and counterregulated *Bcl-2* and *Bax* gene expressions. Although *Creb* and *Bdnf* gene expressions increased with MA, the expressions were not significantly higher than the expressions in the impaired rats. Considering that several previous studies have shown the regulation of CREB and BDNF neurotrophic signaling by MA stimulation of the points GV20 [47] and HT7 [19], the limited upregulation of *Creb* and *Bdnf* gene expressions shown by MA after cognitive impairment in this study was unexpected. One possible explanation for this discrepancy is the difference in the methods used to induce cognitive impairment. While cognitive impairment was induced via MCAO surgery in the present study, in the previous studies, cognitive impairment was induced via repeated administration of either corticosterone [19] or scopolamine [47]. The model-dependent effects of MA on cognitive deficit cannot be completely explained, and further studies are required to elucidate the differences in the effects of MA in diverse cognitive deficit models.

5. Conclusion

In conclusion, the present study confirmed the effects of LA on MCAO with behavioral test in Morris water maze test, ChAT immunoreactivity in the hippocampal CA1 region, and *Creb*, *Bdnf*, *Bcl-2*, and *Bax* gene expressions. It suggests that LA treatment could improve cognitive impairment in MCAO rats to enhance the cholinergic system in the hippocampal CA1 region and to exert a neuroprotective effect by regulating *Creb*, *Bdnf*, *Bcl-2*, and *Bax* gene expressions. The present findings provide evidence for the therapeutic effects of LA on MCAO-induced cognitive impairment. In order to develop treatment standards for the clinical application of LA, subsequent studies should be required for revealing more precisely how these effects of LA depend on acupuncture points and treatment parameters, such as wavelength, power output, frequency, exposure time, and beam profile.

Conflicts of Interest

The authors declare no conflicts of interest, and all authors have approved the final paper.

Authors' Contributions

Yeong-Chan Yun and Dongyeop Jang contributed equally to this work.

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References

[1] T. J. Kaptchuk, "Acupuncture: theory, efficacy, and practice," *Annals of Internal Medicine*, vol. 136, no. 5, pp. 374–383, 2002.

[2] S. Zhang, B. Wu, M. Liu et al., "Acupuncture efficacy on ischemic stroke recovery: multicenter randomized controlled trial in China," *Stroke*, vol. 46, no. 5, pp. 1301–1306, 2015.

[3] Q.-Q. Li, G.-X. Shi, J.-W. Yang et al., "Hippocampal cAMP/PKA/CREB is required for neuroprotective effect of acupuncture," *Physiology and Behavior*, vol. 139, pp. 482–490, 2015.

[4] A.-J. Liu, J.-H. Li, H.-Q. Li et al., "Electroacupuncture for acute ischemic stroke: a meta-analysis of randomized controlled trials," *American Journal of Chinese Medicine*, vol. 43, no. 8, pp. 1541–1566, 2015.

[5] H.-F. Zhang, H.-X. Li, Y.-C. Dai et al., "Electro-acupuncture improves the social interaction behavior of rats," *Physiology and Behavior*, vol. 151, pp. 485–493, 2015.

[6] P. Whittaker, "Laser acupuncture: past, present, and future," *Lasers in Medical Science*, vol. 19, no. 2, pp. 69–80, 2004.

[7] C. Sutalangka, J. Wattanathorn, S. Muchimapura, W. Thukham-Mee, P. Wannanon, and T. Tong-Un, "Laser acupuncture improves memory impairment in an animal model of alzheimer's disease," *Journal of Acupuncture and Meridian Studies*, vol. 6, no. 5, pp. 247–251, 2013.

[8] J. Wattanathorn and C. Sutalangka, "Laser acupuncture at HT7 acupoint improves cognitive deficit, neuronal loss, oxidative stress, and functions of cholinergic and dopaminergic systems in animal model of parkinson's disease," *Evidence-based Complementary and Alternative Medicine*, vol. 2014, Article ID 937601, 8 pages, 2014.

[9] J. Khongrum and J. Wattanathorn, "Laser acupuncture improves behavioral disorders and brain oxidative stress status

in the valproic acid rat model of autism," *Journal of Acupuncture and Meridian Studies*, vol. 8, no. 4, pp. 183–191, 2015.

[10] J. T. O'Brien, T. Erkinjuntti, B. Reisberg et al., "Vascular cognitive impairment," *Lancet Neurology*, vol. 2, no. 2, pp. 89–98, 2003.

[11] T. Sugawara, A. Lewén, N. Noshita, Y. Gasche, and P. H. Chan, "Effects of global ischemia duration on neuronal, astroglial, oligodendroglial, and microglial reactions in the vulnerable hippocampal CA1 subregion in rats," *Journal of Neurotrauma*, vol. 19, no. 1, pp. 85–98, 2002.

[12] T. L. Butler, C. A. Kassed, P. R. Sanberg, A. E. Willing, and K. R. Pennypacker, "Neurodegeneration in the rat hippocampus and striatum after middle cerebral artery occlusion," *Brain Research*, vol. 929, no. 2, pp. 252–260, 2002.

[13] K. Hattori, H. Lee, P. D. Hurn, B. J. Crain, R. J. Traystman, and A. C. DeVries, "Cognitive deficits after focal cerebral ischemia in mice," *Stroke*, vol. 31, no. 8, pp. 1939–1944, 2000.

[14] K. Kitagawa, "CREB and cAMP response element-mediated gene expression in the ischemic brain," *FEBS Journal*, vol. 274, no. 13, pp. 3210–3217, 2007.

[15] B. H. Han, A. D'Costa, S. A. Back et al., "BDNF blocks caspase-3 activation in neonatal hypoxia-ischemia," *Neurobiology of Disease*, vol. 7, no. 1, pp. 38–53, 2000.

[16] J.-C. Martinou, M. Dubois-Dauphin, J. K. Staple et al., "Overexpression of BCL-2 in transgenic mice protects neurons from naturally occurring cell death and experimental ischemia," *Neuron*, vol. 13, no. 4, pp. 1017–1030, 1994.

[17] J. Y. Youn, Y. S. Kim, D. H. Youn et al., "Comparative studies of effects of manual acupuncture, invasive laser acupuncture and laser skin irradiation at the Heart Jeonggyeok acupoint on the focal ischemia induced by intraluminal filament insertion in rats," *Journal of Meridian & Acupoint*, vol. 27, pp. 125–142, 2010.

[18] W.-W. Wang, C.-L. Xie, L. Lu, and G.-Q. Zheng, "A systematic review and meta-Analysis of Baihui (GV20)-based scalp acupuncture in experimental ischemic stroke," *Scientific Reports*, vol. 4, article 3981, 2014.

[19] B. Lee, B.-J. Sur, S. Kwon et al., "Acupuncture stimulation alleviates corticosterone-induced impairments of spatial memory and cholinergic neurons in rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 670536, 14 pages, 2012.

[20] E. Z. Longa, P. R. Weinstein, S. Carlson, and R. Cummins, "Reversible middle cerebral artery occlusion without craniectomy in rats," *Stroke*, vol. 20, no. 1, pp. 84–91, 1989.

[21] World Health Organization, *WHO Standard Acupuncture Point Locations in the Western Pacific Region*, vol. 181, World Health Organization, Geneva, Switzerland, 2008.

[22] C. V. Vorhees and M. T. Williams, "Morris water maze: procedures for assessing spatial and related forms of learning and memory," *Nature Protocols*, vol. 1, no. 2, pp. 848–858, 2006.

[23] V. S. Sierpina and M. A. Frenkel, "Acupuncture: a clinical review," *Southern Medical Journal*, vol. 98, no. 3, pp. 330–337, 2005.

[24] R. Chow, W. Yan, and P. Armati, "Electrophysiological effects of single point transcutaneous 650 and 808nm laser irradiation of rat sciatic nerve: a study of relevance for low-level laser therapy and laser acupuncture," *Photomedicine and Laser Surgery*, vol. 30, no. 9, pp. 530–535, 2012.

[25] I. Quah-Smith, C. Smith, J. D. Crawford, and J. Russell, "Laser acupuncture for depression: a randomised double blind controlled trial using low intensity laser intervention," *Journal of Affective Disorders*, vol. 148, no. 2-3, pp. 179–187, 2013.

[26] R. R. Anderson and J. A. Parrish, "The optics of human skin," *Journal of Investigative Dermatology*, vol. 77, no. 1, pp. 13–19, 1981.

[27] M. J. C. Van Gemert, S. L. Jacques, H. J. C. M. Sterenborg, and W. M. Star, "Skin optics," *IEEE Transactions on Biomedical Engineering*, vol. 36, no. 12, pp. 1146–1154, 1989.

[28] H. M. Langevin, D. L. Churchill, and M. J. Cipolla, "Mechanical signaling through connective tissue: a mechanism for the therapeutic effect of acupuncture," *The FASEB Journal*, vol. 15, no. 12, pp. 2275–2282, 2001.

[29] H. M. Langevin, D. L. Churchill, J. R. Fox, G. J. Badger, B. S. Garra, and M. H. Krag, "Biomechanical response to acupuncture needling in humans," *Journal of Applied Physiology*, vol. 91, no. 6, pp. 2471–2478, 2001.

[30] S. Wan, J. A. Parrish, R. R. Anderson, and M. Madden, "Transmittance of nonionizing radiation in human tissues," *Photochemistry and Photobiology*, vol. 34, no. 6, pp. 679–681, 1981.

[31] M. H. Niemz, *Laser-Tissue Interactions: Fundamentals and Applications*, Springer Science & Business Media, New York, NY, USA, 2013.

[32] J. Zhao, K. Cheng, H. Deng et al., "The effect of different laser irradiation on cyclophosphamide-induced leucopenia in rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 2014, Article ID 756406, 4 pages, 2014.

[33] H. Macpherson, *Acupuncture Research: Strategies for Establishing an Evidence Base*, Elsevier Health Sciences, 2007.

[34] Z. Jonasson, "Meta-analysis of sex differences in rodent models of learning and memory: a review of behavioral and biological data," *Neuroscience and Biobehavioral Reviews*, vol. 28, no. 8, pp. 811–825, 2005.

[35] O. G. Nilsson, R. E. Strecker, A. Daszuta, and A. Björklund, "Combined cholinergic and serotonergic denervation of the forebrain produces severe deficits in a spatial learning task in the rat," *Brain Research*, vol. 453, no. 1-2, pp. 235–246, 1988.

[36] R. L. Roof, G. P. Schielke, X. Ren, and E. D. Hall, "A comparison of long-term functional outcome after 2 middle cerebral artery occlusion models in rats," *Stroke*, vol. 32, no. 11, pp. 2648–2657, 2001.

[37] D. M. Armstrong, C. B. Saper, A. I. Levey, B. H. Wainer, and R. D. Terry, "Distribution of cholinergic neurons in rat brain: deomonstrated by the immunocytochemical localization of choline acetyltransferase," *Journal of Comparative Neurology*, vol. 216, no. 1, pp. 53–68, 1983.

[38] G. Chen, K. S. Chen, J. Knox et al., "A learning deficit related to age and β-amyloid plaques in a mouse model of alzheimer's disease," *Nature*, vol. 408, no. 6815, pp. 975–979, 2000.

[39] C. Martin-Ruiz, J. Court, M. Lee et al., "Nicotinic receptors in dementia of Alzheimer, lewy body and vascular types," *Acta Neurologica Scandinavica, Supplement*, vol. 102, no. 176, pp. 34–41, 2000.

[40] H. Lshimaru, A. Takahashi, Y. Ikarashi, and Y. Maruyama, "Immunohistochemical and neurochemical studies of hippocampal cholinergic neurones after ischaemia," *NeuroReport*, vol. 6, no. 3, pp. 557–560, 1995.

[41] T. Hara, J.-I. Hamada, S. Yano, M. Morioka, Y. Kai, and Y. Ushio, "CREB is required for acquisition of ischemic tolerance in gerbil hippocampal CA1 region," *Journal of Neurochemistry*, vol. 86, no. 4, pp. 805–814, 2003.

[42] X. Tao, S. Finkbeiner, D. B. Arnold, A. J. Shaywitz, and M. E. Greenberg, "Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism," *Neuron*, vol. 20, no. 4, pp. 709–726, 1998.

[43] B. R. S. Broughton, D. C. Reutens, and C. G. Sobey, "Apoptotic mechanisms after cerebral ischemia," *Stroke*, vol. 40, no. 5, pp. e331–e339, 2009.

[44] M. D. Linnik, R. H. Zobrist, and M. D. Hatfield, "Evidence supporting a role for programmed cell death in focal cerebral ischemia in rats," *Stroke*, vol. 24, no. 12, pp. 2002–2008, 1993.

[45] F. Gillardon, C. Lenz, K. F. Waschke et al., "Altered expression of Bcl-2, Bcl-X, Bax, and c-Fos colocalizes with DNA fragmentation and ischemic cell damage following middle cerebral artery occlusion in rats," *Molecular Brain Research*, vol. 40, no. 2, pp. 254–260, 1996.

[46] M. C. P. Leung, K. K. Yip, Y. S. Ho, F. K. W. Siu, W. C. Li, and B. Garner, "Mechanisms underlying the effect of acupuncture on cognitive improvement: a systematic review of animal studies," *Journal of Neuroimmune Pharmacology*, vol. 9, no. 4, pp. 492–507, 2014.

[47] B. Lee, B. Sur, J. Shim, D.-H. Hahm, and H. Lee, "Acupuncture stimulation improves scopolamine-induced cognitive impairment via activation of cholinergic system and regulation of BDNF and CREB expressions in rats," *BMC Complementary and Alternative Medicine*, vol. 14, no. 1, article 338, 2014.

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Research Article

Effects of a Herbal Medicine, Yukgunja-Tang, on Functional Dyspepsia Patients Classified by 3-Dimensional Facial Measurement: A Study Protocol for Placebo-Controlled, Double-Blind, Randomized Trial

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Introduction. Functional dyspepsia (FD), a common upper gastrointestinal disease, is difficult to manage because of the limitations of current conventional treatments. Yukgunja-tang (YGJT) is widely used to treat FD in clinical practice in Korea, Japan, and China. However, YGJT significantly improves few symptoms of FD. In Korean medicine, FD is a well-known functional gastric disease that shows difference in the effect of herbal medicine depending on constitution or type of Korean medicine diagnosis. This study aims to investigate the efficacy of YGJT on FD patients classified by 3-dimensional facial measurement using a 3-dimensional facial shape diagnostic system (3-FSDS). *Methods*. A placebo-controlled, double-blind, randomized, two-center trial will be performed to evaluate the efficacy of YGJT on FD patients. Eligible subjects will be initially classified as two types by 3-dimensional facial measurement using the 3-FSDS. Ninety-six subjects (48 subjects per each type) will be enrolled. These subjects will be randomly allocated into treatment or control groups in a 2 : 1 ratio. YGJT or placebo will be administered to each group during the 8-week treatment period. The primary outcome is total dyspepsia symptom scale, and the secondary outcomes include single dyspepsia symptom scale, proportion of responders with adequate symptom relief, visual analog scale, Nepean dyspepsia index-Korean version, functional dyspepsia-related quality of life, and spleen qi deficiency questionnaire. *Discussion*. This is the first randomized controlled trial to assess the efficacy of the YGJT on FD patients classified by 3-dimensional facial measurement. We will compare the treatment effect of the YGJT on FD patients classified as two types using the 3-FSDS. The results of this trial will help the FD patients improve the symptoms and quality of life effectively and provide objective evidence for prescribing the YGJT to FD patients in clinical practice. *Trial Registration*. This trial is registered with Clinical Research Information Service Identifier: KCT0001920, 15 May, 2016.

1. Introduction

Functional dyspepsia (FD) is a common functional gastrointestinal disorder characterized by chronic or recurrent abdominal discomfort or pain and symptoms, such as epigastric pain, epigastric burning, postprandial discomfort, and early satiety without evidence of organic diseases as confirmed by esophagogastroduodenoscopy (EGD) [1]. The prevalence of FD ranges from 11% to 29.2% worldwide [2]. In particular, more than 40% of patients who visit primary clinics and tertiary hospitals in South Korea have been diagnosed as having FD according to a survey using the Rome III criteria [3]. Various therapies for FD, including dietary modifications, antiemetics, antispasmodics, prokinetics, and analgesics, are commonly used as conventional treatments [4]. However, many patients with FD often seek

out complementary and alternative therapies, such as herbal medicines and acupuncture, because of the limited effects of the conventional therapies [5–7].

Yukgunja-tang (YGJT; Rikkunshito in Kampo Medicine; Liu Jun Zi Tang in Traditional Chinese Medicine) is a herbal medicine comprising eight herbs; it is used to treat FD and relieve upper gastrointestinal symptoms, including dyspepsia, epigastric discomfort, and anorexia, in clinical practice in Korea, Japan, China, and other Asian areas [8–11]. In animal studies, YGJT improves relaxation of the gastric fundus, which maintains gastric storage capacity, and enhances gastric antral peristalsis, which facilitates stomach emptying. These effects improve gastric accommodation and emptying [12–16]. Some randomized controlled trials have been conducted to investigate the effect of YGJT on FD [17–21]. However, YGJT significantly improves only some symptoms of FD [18, 20]. In Korean medicine, FD is a common gastrointestinal disease that shows different treatment effects of herbal medicine depending on specific constitution or type of Korean medicine diagnosis. Accordingly, we will perform a randomized controlled trial to evaluate the efficacy of YGJT on FD patients classified as specific type by 3-dimensional facial measurement, which is one of the diagnostic methods of facial shape diagnosis in Hyungsang medicine.

Hyungsang medicine is a field of contemporary Korean medicine. It is based on the medical theories of Donguibogam, one of the most influential texts in Korean medicine. Hyungsang medicine emphasizes the fundamental constituents of humans and their constitutional differences and classifies persons into several types according to their outer appearances (shapes and colors) [22]. Facial shape diagnosis, a part of Hyungsang medicine, is conducted by observing facial characteristics like the shape and area of the face, along with the shape and location of the ear, eye, mouth, and nose. Facial shape diagnosis can be classified into several types including a bladder and a gallbladder body according to the aforementioned facial characteristics. For instance, people classified as bladder body have a wider frontal part of the face than the side part, and an overall round face and relatively big mouth. People classified as gallbladder body have a wider side part of the face than the frontal part, along with an angular face and relatively big nose [23, 24]. The theory of Hyungsang medicine states that if patients' outer appearances are different, the status of their inner organs is also different; therefore, even if the outer symptoms are the same, the treatments are different [22]. For example, YGJT, which will be used in this trial, is known to be more effective for the same symptoms in patients classified as the bladder than the gallbladder body [23, 25]. However, facial shape diagnosis, which is an important factor in Hyungsang medicine, has limitation; that is, it is performed through direct observation by clinicians. Thus, the diagnostic result tends to be subjective and inconsistent. The importance of a diagnostic device that can overcome the above limitation has recently been the focus to achieve more objective and standardized diagnosis [24, 26].

A 3-dimensional facial shape diagnostic system (3-FSDS) is a device for 3-dimensional facial measurement, and it is developed to overcome the limitations of the existing methods for the facial shape diagnosis. This device quantitatively measures the facial characteristics including the shape and area of the face, along with the shape and location of the ear, eye, mouth, and nose, and objectively classifies the type of facial shape as the bladder or the gallbladder body. The 3-FSDS is produced by Morpheus Co., Ltd. (Seongnam, Korea), with product manufacture approval from Korea Food & Drug Administration. Several studies have reported the development and clinical application of the 3-FSDS for 3-dimensional facial measurement [24, 27–31].

In this study, we will conduct a placebo-controlled, double-blind, randomized, two-center trial to demonstrate the efficacy of YGJT on FD patients classified as either bladder or gallbladder body by 3-dimensional facial measurement using the 3-FSDS and to verify the usefulness of the 3-FSDS for 3-dimensional facial measurement.

2. Materials and Methods

2.1. Study Design. This study will be conducted as a placebo-controlled, double-blind, randomized, two-center trial at the Kyung Hee University Korean Medicine Hospital in Seoul and the Dong-Eui University Korean Medicine Hospital in Busan, Korea, from July 2016 to November 2017.

The trial will comprise a 1-week run-in period (week −1 to 0) and an 8-week treatment period (week 0 to 8). After screening and eligibility tests, subjects will be classified as specific type ("bladder body" or "gallbladder body") using the 3-FSDS. A total of 96 subjects (48 bladder body, 48 gallbladder body) will be enrolled. The subjects will be randomized to treatment or control groups in a 2 : 1 ratio, including the same number of each subject type in each group, by an independent statistician. During the treatment period, 5.0 g of YGJT or placebo will be provided thrice a day (1 hour after each meal) for 8 weeks to the treatment or control groups, respectively. The trial flow is shown in Figure 1.

2.2. Participants

2.2.1. Inclusion Criteria. Subjects who meet the following will be included: (1) subjects aged 19–75 years; (2) subjects who meet the Rome III criteria for FD; (3) subjects with more than 40 points on the visual analog scale (VAS; 0, no discomfort; 100, most severe discomfort) for the severity of dyspeptic symptoms; (4) subjects who agree to receive no other treatments during the study; (5) subjects who voluntarily agree with the study protocol and sign a written informed consent.

2.2.2. Exclusion Criteria. Subjects who report any of the following will be excluded: (1) subjects with peptic ulcer or gastroesophageal reflux disease confirmed on EGD; (2) subjects with obvious signs of irritable bowel syndrome; (3) subjects with alarm symptoms, such as severe weight loss, melena, and dysphagia; (4) subjects with severe systemic organ diseases (cancer, diseases of heart, lung, liver, or kidney) or mental illness; (5) subjects who have had surgery related to the gastrointestinal tract, except for appendectomy more than six months ago; (6) subjects taking drugs that

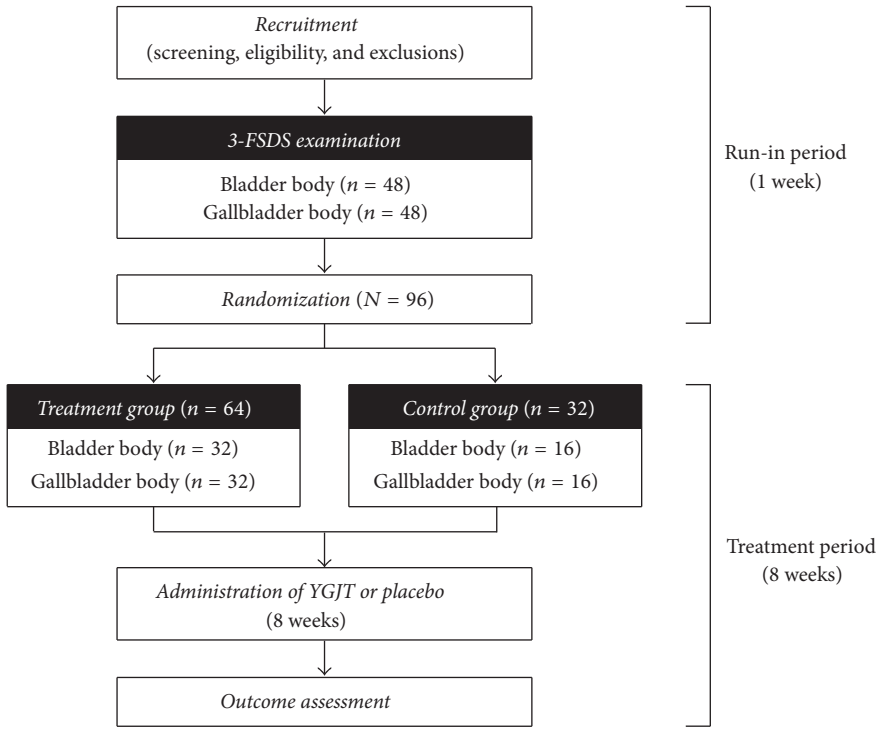


Figure 1: Flow chart of the trial. 3-FSDS, 3-dimensional facial shape diagnostic system; YGJT, Yukgunja-tang.

might affect the gastrointestinal tract; a minimum washout period of a week is required before participating in the study; (7) subjects who have had maxillofacial surgery or facial bone contouring surgery; (8) subjects who are pregnant or breastfeeding; (9) subjects who have malabsorption or maldigestion; (10) HIV positive subjects; (11) subjects with difficulties in taking part in the study (e.g., serious mental illness, dementia, drug addiction, time constraint, severe disorder in vision or hearing, and illiteracy); (12) subjects who have taken investigational drugs for other trials in the last three months.

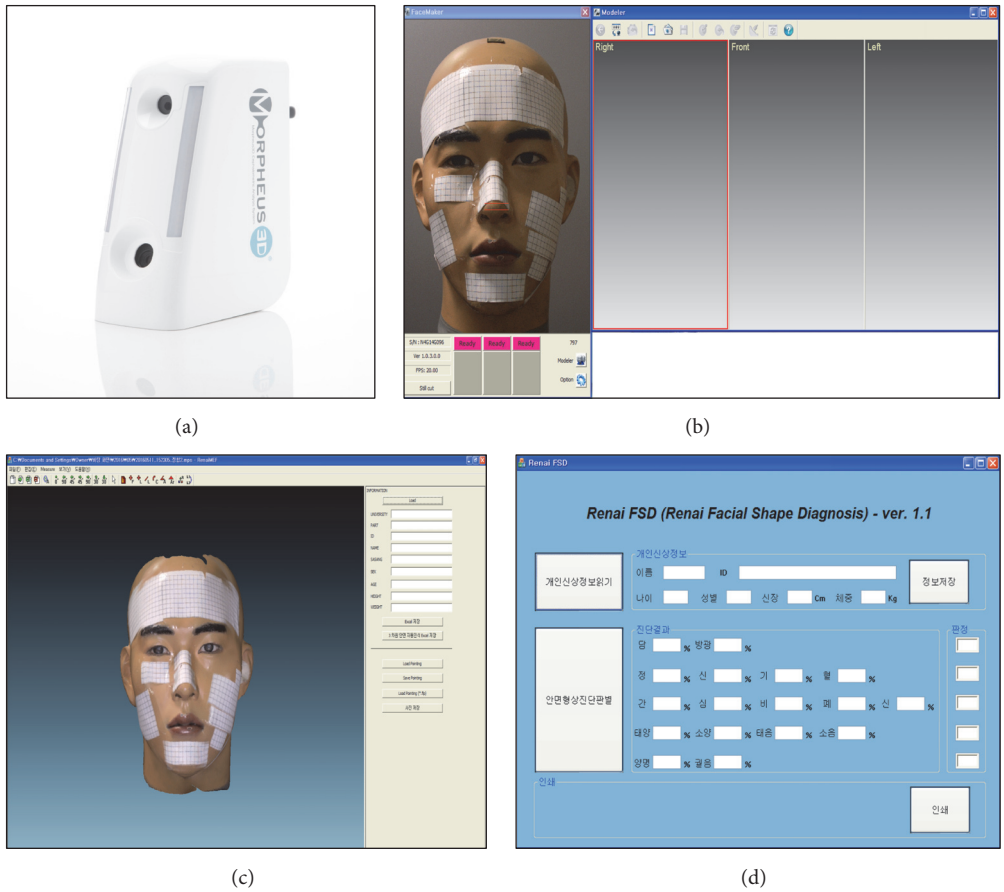
2.3. Recruitment. Advertisements will be placed on the notice boards and homepages of the Kyung Hee University Korean Medicine Hospital and the Dong-Eui University Korean Medicine Hospital. The purpose, procedures, and potential risks and benefits of the study will be provided to the subjects in detail. All subjects should voluntarily sign written informed consent forms prior to enrollment.

2.4. Randomization, Allocation Concealment, and Blinding. Randomization will be separately performed at each center by an independent statistician. The subjects will be randomly assigned to the treatment or control group in a 2 : 1 ratio using block randomization method. This ratio is chosen because it will be more ethical to assign twice the number of subjects who will take placebo to the treatment group than to allocate equal number of subjects to each group [17]. Random numbers will be generated using the PROC PLAN of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) by the independent statistician. The statistician will arrange the random numbers of each center in the two types (bladder body, gallbladder body) of facial shape and send the allocation lists to an independent staff. The staff will be in charge of the 3-FSDS program operation and know the type of facial shape of each subject.

When a subject passes the screening and eligibility test, his/her face image will be taken and stored using the 3-FSDS. Subsequently, the image will be sent to the independent staff operating the 3-FSDS program; the type of facial shape will be classified by 3-dimensional facial measurement using the 3-FSDS program. After the type is determined, the staff will immediately inform an investigator of the center through e-mail whether the subject will be included in the trial considering the fixed number of subjects for each type (24 bladder body, 24 gallbladder body at each center). If the subject is included in the trial, the staff will send a random number in the allocation list arranged in sequential order in each type to the investigator of the center through e-mail. Additionally, if the fixed number of subjects of each type is already full, the staff will notify the investigator that the new subject will be excluded from the trial.

The subjects, investigators, clinical research coordinator (CRC), and clinical pharmacist will be blinded to the random allocation of the trial. Only the independent statistician will be associated with the randomization. The independent staff operating the 3-FSDS program will know only the type of facial shape not the group allocation of each subject.

2.5. 3-Dimensional Facial Shape Diagnostic System (3-FSDS). The 3-FSDS comprises a 3D facial scanner (Morpheus 3D), scanner driving and data generation program (Real Face),



(a) (b) (c) (d)

Figure 2: Components of 3-dimensional facial shape diagnostic system (3-FSDS). (a) Morphues 3D, (b) Real Face, (c) Renai MEF, and (d) Renai FSD.

3D facial shape measurement program (Renai MEF), and 3D facial shape diagnosis program (Renai FSD) (Figure 2). The Morpheus 3D (Figure 2(a)) is a scanner for the 3D facial shape and is equipped with a camera (1024 ∗ 768, MV-CX37U, CREVIS), white LED light (5500 K), pattern generator, projector, and cooling fan. This scanner is convenient to operate and can capture 3D facial shape with a short scan time of only 0.8 s. It is safe to use because it does not harm the eyes [27]. The Real Face program (Figure 2(b)) controls the camera and the projector of the Morpheus 3D and captures the patterned projected images continuously at a high speed. The Real Face generates 3D data with acquired images through spatial coding, noise reduction filtering, 3D coordinate generations, mesh generations, and texture coordinate generations. The Renai MEF program (Figure 2(c)) automatically detects feature points and acquires the 3D coordinates of the points (including 39 points in the frontal part and 15 points in the side part of the face) from the 3D data. This program measures the length between the two points, angle of the three points, and area of the polygon on the eye, eyebrow, nose, and mouth parts with the images acquired by the Real Face and generates 337 variables. The Renai FSD program (Figure 2(d)) classifies the types of facial shape as the bladder or the gallbladder body after analyzing the variables obtained from the Renai MEF and calculating discriminant function. The analysis result is presented as percentage at each type, and the type with higher probability is considered as the final result of 3-dimensional facial measurement.

2.6. Interventions. After randomization, all subjects will be divided into the treatment (YGJT group) and control groups (placebo group). Each group will be provided with YGJT or placebo; the 5.0 g of YGJT or placebo will be taken thrice a day during the 8-week treatment period.

Brown bitter herbal extract YGJT granules (Yukgunja-tang granule®, Hankookshinyak Co., Ltd., Nonsan, Korea) produced in accordance with the Korean Good Manufacturing Practice guidelines will be used in this trial. Yukgunja-tang granule, a water-extracted YGJT mixed with cornstarch and lactose, is permitted and regulated by the Korean Food & Drug Administration. Each 5.0 g of YGJT granule contains *Pinelliae tuber* (1.33 g), *Citri unshii pericarpium* (1.33 g), Ginseng Radix Alba (1.33 g), Atractylodis Rhizoma Alba (1.33 g), Hoelen (1.33 g), *Glycyrrhizae radix* (0.50 g), Zingiberis Rhizoma Crudus (0.67 g), and *Zizyphi fructus* (0.67 g) as raw materials. All herbs will be obtained from qualified suppliers in Korea, and the YGJT granules will be sealed in opaque aluminum bags.

The placebo YGJT will be made with cornstarch and lactose with the same color and taste as the real YGJT

by Hankookshinyak Co., Ltd., using the standard method of placebo manufacturing according to the Korean Good Manufacturing Practice guidelines. The placebo YGJT will be packed identically to the real YGJT in opaque aluminum bags with the same labeling.

The independent clinical pharmacist will distribute the investigational drugs to the subjects, and the CRC will ensure correct distribution. The subjects will be instructed to dissolve the YGJT or placebo granules in water and take them 1 hour after meals. The subjects are required to return the unused investigational drugs to the CRC at the next visit. The CRC and the clinical pharmacist will check the number of the returned investigational drugs and record it on a case report form (CRF). Treatment compliance will be evaluated at the end of the study by counting the number of the returned unused YGJT or placebo. The subjects with less than 70% compliance will be excluded from *per-protocol* analysis.

The subjects will not be allowed to take any concomitant medications associated with the treatment of FD during the trial. The subjects will also be instructed to report all prescribed or over-the-counter medications taken during the study at each visit and each telephone visit.

2.7. Outcome Measurements

2.7.1. Primary Outcome

Total Dyspepsia Symptom Scale (TDS Scale). The TDS scale comprises 8 items (postprandial fullness and bloating, early satiety, epigastric pain, epigastric burning, nausea, vomiting, belching, and other symptoms), with a 4-point Likert scale [17, 32]. The TDS score is the total score of 8 items. This scale will be assessed at baseline, 4 weeks, and 8 weeks.

2.7.2. Secondary Outcomes

(1) Single Dyspepsia Symptom Scale (SDS Scale). The SDS scale comprises three aspects of four principal symptoms of FD with a 4-point Likert scale [17, 32]. The symptoms are epigastric pain, epigastric burning, postprandial fullness and bloating, and early satiety. The three aspects are the frequency, intensity, and level of discomfort. The SDS score is the total score of the three aspects of the four symptoms. This scale will be evaluated at baseline, 4 weeks, and 8 weeks.

(2) Adequate Relief (AR) of FD Pain and Discomfort. The AR will be measured to assess the weekly improvement of the overall FD symptoms at each visit and each telephone visit during the treatment period. Subjects will be asked to answer the following question: "In the last week, have you had adequate relief of your pain or discomfort related to FD?" A proportion of responders (PR) will be assessed to compare the efficacy of the treatment. The responders will be defined as subjects reporting adequate relief for at least 50% of the treatment period, that is, responding "Yes" more than four times out of eight.

(3) Visual Analogue Scale (VAS). The VAS measures the severity of overall dyspeptic symptoms (ranging from 0 mm as no discomfort to 100 mm as the most severe discomfort). The VAS will be measured at baseline, 4 weeks, and 8 weeks.

(4) Nepean Dyspepsia Index-Korean Version (NDI-K). The Nepean dyspepsia index (NDI) [33, 34] is a reliable and validated disease-specific index for FD, which measures symptoms and health-related quality of life. The Korean version of NDI (NDI-K) validated by Lee et al. will be used [35, 36]. In this study, symptom-based questions, including the period, severity, and degree of distress of 15 symptoms, will be assessed using a 5- or 6-point Likert scale at baseline, 4 weeks, and 8 weeks.

(5) Functional Dyspepsia-Related Quality of Life (FD-QoL) Questionnaire. The FD-QoL questionnaire measures the quality of life of FD patients. This questionnaire comprises four subscales of diet (5 items), daily activity (4 items), emotion (6 items), and social functioning (6 items) with a 5-point Likert scale. Higher total sum scores indicate worse quality of life. The FD-QoL questionnaire will be evaluated at baseline, 4 weeks, and 8 weeks.

(6) Spleen Qi Deficiency Questionnaire (SQDQ). The SQDQ will be used to evaluate the spleen qi deficiency syndrome, which is the most common syndrome in FD patients [37, 38]. The questionnaire developed by Oh et al. [39] is composed of 11 items, and the total sum scores will be calculated weighing for each symptom. A cut-off point of the SQDQ to determine whether the subject is in a condition of spleen qi deficiency is 43.18 [40]. The SQDQ will be assessed at baseline, 4 weeks, and 8 weeks.

2.8. Safety and Adverse Event. The following tests will be performed to assess the safety at screening and 8 weeks: white blood cell, red blood cell, hemoglobin, hematocrit, platelet, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, blood urea nitrogen, creatinine, and erythrocyte sedimentation rate. These tests will enable the investigators to exclude subjects with serious diseases before randomization and to verify the safety of the 8-week administration of YGJT in FD patients.

Adverse events (AEs) including serious AEs will be reported and recorded in detail during the entire study. An AE is any untoward medical occurrence that will not necessarily have a causal relationship with an investigational drug. Therefore, an AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational drug, whether or not related to those. If any AEs occur, the appropriate treatment will be provided to the subject immediately. A serious AE will be defined as an event resulting in death, a life-threatening event, an illness requiring hospitalization, or persistent or significant disability. All of the serious AEs will be promptly reported to the institutional review board and the principal investigator within 24 hours.

2.9. Sample Size Calculation. The formula for estimating the sample size is as follows:

$$N = n_t + n_c \quad (n_t, \text{the number of treatment group}; n_c, \text{the number of control group})$$
$$n_c = \frac{1}{2} n_t = \frac{\left\{\left(Z_{\alpha/2} + Z_\beta\right)^2 \sigma^2 (\lambda + 1)/\lambda\right\}}{\left(\mu_c - \mu_t\right)^2}. \quad (1)$$

A previous study demonstrated 1.57 points of improvement ($\mu_c - \mu_t = \delta$) in the TDS scale for FD after 4 weeks of YGJT treatment compared with that after the placebo treatment [17]. The study indicated a mean standard deviation (SD = σ) of 2.148. In the present study, the ratio (λ) of the treatment group to the control group will be 2 : 1 ($\lambda = 2$). With a power ($1 - \beta$) of 80% and a significance level (α) of 5%, assuming $\delta = 1.57$ and $\sigma = 2.148$, a sample size of $n_t = 44$ and $n_c = 22$ subjects will be required. Considering an assumed dropout rate of 30%, a total of 96 subjects will be needed in the current study.

2.10. Statistical Analysis. An independent statistician who is blinded to group allocation will conduct statistical analysis. All data will be presented as mean ± standard deviation or number (%). Baseline characteristics of the subjects will be analyzed using chi-squared test or Fisher's exact test for categorical variables and analysis of variance (ANOVA) for continuous variables. For the efficacy analysis, two-way ANOVA will be used to compare the changes in scores of each outcome for 8 weeks between the intervention groups or the facial shape types. For the safety analysis, the chi-squared test or Fisher's exact test will be used. Both *intention-to-treat* (ITT) and *per-protocol* (PP) analyses will be performed in the current trial, and the primary analysis will be based on the ITT analysis. For the ITT analysis, a full analysis set will be used, and all subjects randomly allocated to one of the two groups, have taken at least one dose of the investigational drugs, and have reported once at least the primary outcome after the baseline will be included. Missing data will be adjusted using the last observation carried forward method. For the PP analysis, all subjects who have completed the study and have complied well with the study protocol without major protocol violations will be included. The subjects with <70% compliance will be excluded from the PP analysis. For the safety analysis, all subjects who have taken at least one dose of the investigational drugs will be included. SPSS 21.0 (IBM SPSS Statistics, New York, USA) will be used for all statistical analyses. A P value < .05 will be considered statistically significant.

2.11. Quality Control. Regular monitoring will be conducted at each center by checking trial master files, informed consent forms, CRFs, compliance with treatments, AEs, and data records to ensure the accuracy and quality.

2.12. Ethics. The current study will be conducted in accordance with the standards of the International Committee on Harmonization of Good Clinical Practice and the revised version of the Declaration of Helsinki. The Institutional Review Board of the Kyung Hee University Korean Medicine Hospital (IRB number KOMCIRB-160115-HR-001) and the Dong-Eui University Korean Medicine Hospital (IRB number 2016-01) approved the trial protocol. This trial is registered at the Clinical Research Information Service (CRIS; KCT0001920; http://cris.nih.go.kr/cris/en/). Written informed consent will be obtained from all subjects prior to enrollment.

3. Discussion

This paper describes the protocol of a trial to investigate the efficacy of YGJT on FD patients classified by 3-dimensional facial measurement. This study is the first to explore the difference of the treatment effect of YGJT on FD patients classified as different types of facial shape using the 3-FSDS.

YGJT has widely been used to treat dyspeptic symptom of upper gastrointestinal diseases including FD in clinical practice in Korea, Japan, and China [8–10, 12]. A few animal and human studies have demonstrated that YGJT is effective on symptoms of FD. However, YGJT is reported to significantly improve only some symptoms of FD in clinical trials. Suzuki et al. [18] conducted a multicenter, double-blind, randomized, placebo-controlled, parallel-group trial to assess the efficacy and safety of YGJT on 247 FD patients. They showed that epigastric pain, an FD symptom, is significantly reduced by administration of YGJT. However, no significant differences were found on the proportion of patients with relief of other symptoms, such as epigastric burning, postprandial fullness, and early satiation, between the YGJT and placebo groups. In addition, a clinical trial with FD patients performed by Kusunoki et al. [20] reported that although the total score of the gastrointestinal symptom rating scale decreases after treatment with YGJT, the differences are not statistically significant. This finding may be caused by the treatment of FD, which is affected by specific constitution and type of Korean medicine diagnosis of patients. Differences of treatment effect of identical herbal medicine between FD patients with different constitutions or diagnosis types are possible. In traditional Korean medicine, particular facial characteristics have been believed to refer to assessable information related to an individual's diseases, and Hyungsang medicine has been developed as a constitutional medicine based on a Korean classical medicine literature, Donguibogam. According to the theory of Hyungsang medicine, the bladder body type has deficient qi and excessive dampness; on the other hand, the gallbladder body type has deficient Yin-blood and excessive

heat. Therefore, YGJT is considered as a herbal medicine which is more effective to the bladder body type [22, 41]. Accordingly, we will examine the efficacy of YGJT on FD patients classified as bladder or gallbladder body by 3-dimensional facial measurement using the 3-FSDS.

In addition, we will evaluate the usefulness of the 3-FSDS comparing the treatment effect of YGJT, which is more effective on patients classified as the bladder than the gallbladder body in the theory of Hyungsang medicine, for FD patients classified as different facial shape types using the 3-FSDS. As mentioned above, the 3-FSDS is a diagnostic device developed to overcome the limitations of the existing methods for the facial shape diagnosis. If the symptoms of FD in this trial more significantly improve patients classified using the 3-FSDS as the bladder than the gallbladder body, then the 3-FSDS can be used to classify the different types of facial shape by 3-dimensional facial measurement.

The results of the current study are expected to demonstrate whether YGJT administration shows difference of treatment effect on FD patients depending on the facial shape types classified as bladder or gallbladder body using the 3-FSDS. If the efficacy of YGJT on FD patients classified as specific type is proven in this trial, their dyspeptic symptoms and quality of life will improve more effectively. The objective evidence for prescribing YGJT to FD patients diagnosed as specific facial shape type will also be provided with more confidence in clinical practice. In addition, if the usefulness of the 3-FSDS is verified, the 3-FSDS will be widely used in clinical practice as a device to diagnose facial shape type by 3-dimensional facial measurement more objectively. Furthermore, more objective diagnosis will aid in achieving a more effective treatment.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Juyeon Kim, Jae-Woo Park, Seok-Jae Ko, Soo-Hyung Jeon, Jong-Won Kim, and Jinsung Kim were responsible for conception and design, data collection and analysis, manuscript writing and critical revision, and final approval of the manuscript; Inkwon Yeo was responsible for statistical design, data analysis and interpretation, and critical revision and final approval of the manuscript. All authors read and approved the final manuscript before submission.

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References

[1] J. Tack and N. J. Talley, "Functional dyspepsia—symptoms, definitions and validity of the Rome III criteria," *Nature Reviews Gastroenterology and Hepatology*, vol. 10, no. 3, pp. 134–141, 2013.

[2] S. Mahadeva and K.-L. Goh, "Epidemiology of functional dyspepsia: a global perspective," *World Journal of Gastroenterology*, vol. 12, no. 17, pp. 2661–2666, 2006.

[3] H. Lee, H.-K. Jung, and K. C. Huh, "Current status of functional dyspepsia in Korea," *The Korean Journal of Internal Medicine*, vol. 29, no. 2, pp. 156–165, 2014.

[4] B. E. Lacy, N. J. Talley, G. R. Locke III et al., "Review article: current treatment options and management of functional dyspepsia," *Alimentary Pharmacology and Therapeutics*, vol. 36, no. 1, pp. 3–15, 2012.

[5] E. Lahner, S. Bellentani, R. D. Bastiani et al., "A survey of pharmacological and nonpharmacological treatment of functional gastrointestinal disorders," *United European Gastroenterology Journal*, vol. 1, no. 5, pp. 385–393, 2013.

[6] P. Moayyedi, S. Soo, J. Deeks, B. Delaney, M. Innes, and D. Forman, "Pharmacological interventions for non-ulcer dyspepsia," *Cochrane Database of Systematic Reviews*, no. 4, 2006.

[7] H. Ouyang and J. D. Z. Chen, "Review article: therapeutic roles of acupuncture in functional gastrointestinal disorders," *Alimentary Pharmacology and Therapeutics*, vol. 20, no. 8, pp. 831–841, 2004.

[8] S. Mogami and T. Hattori, "Beneficial effects of rikkunshito, a Japanese Kampo medicine, on gastrointestinal dysfunction and anorexia in combination with western drug: a systematic review," *Evidence-based Complementary and Alternative Medicine*, vol. 2014, Article ID 519035, 7 pages, 2014.

[9] Y. Xiao, Y.-Y. Liu, K.-Q. Yu, M.-Z. Ouyang, R. Luo, and X.-S. Zhao, "Chinese herbal medicine Liu Jun Zi Tang and Xiang Sha Liu Jun Zi Tang for functional dyspepsia: meta-analysis of randomized controlled trials," *Evidence-based Complementary and Alternative Medicine*, vol. 2012, Article ID 936459, 7 pages, 2012.

[10] Y. Saegusa, T. Hattori, M. Nahata, C. Yamada, and H. Takeda, "A new strategy using rikkunshito to treat anorexia and gastrointestinal dysfunction," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 364260, 10 pages, 2015.

[11] M. Tatsuta and H. Iishi, "Effect of treatment with liu-jun-zi-tang (TJ-43) on gastric emptying and gastrointestinal symptoms in dyspeptic patients," *Alimentary Pharmacology and Therapeutics*, vol. 7, no. 4, pp. 459–462, 1993.

[12] H. Suzuki, J. M. Inadomi, and T. Hibi, "Japanese herbal medicine in functional gastrointestinal disorders," *Neurogastroenterology and Motility*, vol. 21, no. 7, pp. 688–696, 2009.

[13] M. Yanai, E. Mochiki, A. Ogawa et al., "Intragastric administration of rikkunshito stimulates upper gastrointestinal motility and gastric emptying in conscious dogs," *Journal of Gastroenterology*, vol. 48, no. 5, pp. 611–619, 2013.

[14] K. Tominaga, T. Kido, M. Ochi et al., "The traditional Japanese medicine rikkunshito promotes gastric emptying via the antagonistic action of the 5-HT3 Receptor Pathway in Rats," *Evidence-based Complementary and Alternative Medicine*, vol. 2011, Article ID 248481, 8 pages, 2011.

[15] M. Shiratori, T. Shoji, M. Kanazawa, M. Hongo, and S. Fukudo, "Effect of rikkunshito on gastric sensorimotor function under distention," *Neurogastroenterology & Motility*, vol. 23, no. 4, p. 323–e156, 2011.

[16] N. Furukawa, N. Manabe, Y. Kase et al., "Intragastric infusion of rikkunshito (kampo) induces proximal stomach relaxation in conscious dogs," *Autonomic Neuroscience*, vol. 179, no. 1-2, pp. 14–22, 2013.

[17] S. Zhang, L. Zhao, H. Wang et al., "Efficacy of modified LiuJunZi decoction on functional dyspepsia of spleen-deficiency and qi-stagnation syndrome: a randomized controlled trial," *BMC Complementary and Alternative Medicine*, vol. 13, article 54, 2013.

[18] H. Suzuki, J. Matsuzaki, Y. Fukushima et al., "Randomized clinical trial: rikkunshito in the treatment of functional dyspepsia-a multicenter, double-blind, randomized, placebo-controlled study," *Neurogastroenterology and Motility*, vol. 26, no. 7, pp. 950–961, 2014.

[19] M. Arai, T. Matsumura, N. Tsuchiya et al., "Rikkunshito improves the symptoms in patients with functional dyspepsia, accompanied by an increase in the level of plasma ghrelin," *Hepato-Gastroenterology*, vol. 59, no. 113, pp. 62–66, 2012.

[20] H. Kusunoki, K. Haruma, J. Hata et al., "Efficacy of Rikkunshito, a traditional Japanese medicine (Kampo), in treating functional dyspepsia," *Internal Medicine*, vol. 49, no. 20, pp. 2195–2202, 2010.

[21] K. Togawa, J. Matsuzaki, M. Kobayakawa et al., "Association of baseline plasma des-acyl ghrelin level with the response to rikkunshito in patients with functional dyspepsia," *Journal of Gastroenterology and Hepatology (Australia)*, vol. 31, no. 2, pp. 334–341, 2016.

[22] T. Kim, "Classical texts in the present tense: the looking diagnosis of a donguibogam school in South Korea," *The Journal of Alternative and Complementary Medicine*, vol. 20, no. 4, pp. 300–304, 2014.

[23] J. W. Kim, H. C. Kim, Y. T. Lee et al., "Study on diagnosis by facial shapes and sign as a disease-prediction data for a construction of the ante-disease pattern diagno-therapeutic system—focusing on gallbladder's versus bladder's body and masculine versus feminine shape," *Korean Journal of Oriental Physiology & Pathology*, vol. 23, pp. 540–547, 2009.

[24] G. C. Kim, J. W. Lee, H. Kim et al., "Basic study on the image instrument of the facial-form by the 3D-facial scanner," *Korean Journal of Oriental Physiology & Pathology*, vol. 22, pp. 497–501, 2008.

[25] K. Kang, G. Baek, K. Kim, and Y. T. Lee, "Study on the terms, 'Dam' and 'Bangwang,'" *Korean Journal of Oriental Physiology & Pathology*, vol. 17, pp. 275–292, 2003.

[26] J. Seo, Y. Park, and Y. Park, "Review on Hyungsang medicine," *Journal of Korean Medical Science*, vol. 34, pp. 52–68, 2013.

[27] K. K. Kim, Y. T. Lee, J. W. Kim et al., "A study on the number of medical specialist based on the diagnosis correct rate and diagnosis success rate in developing diagnosis function of oriental medicine," *Journal of the Korean Data Analysis Society*, vol. 13, pp. 2853–2865, 2011.

[28] K. K. Kim, J. W. Kim, Y. T. Lee et al., "Study on the statistical methods for the 3D facial data in the Hyungsang clinical medicine-focused on the dam (gall bladder) and bangkwang (urinary bladder) body," *Journal of the Korean Data Analysis Society*, vol. 10, pp. 1327–1337, 2008.

[29] A. Jang, S. Jeon, S. Park et al., "A study on the relationship between Hyungsang classification and Sasang constitutional diagnosis," *Journal of Sasang Constitutional Medicine*, vol. 22, no. 3, pp. 75–86, 2010.

[30] J. W. Kim, K. K. Kim, G. C. Kim et al., "Significance test of facial measuring variable using 3D facial analysis machine," *Journal of the Korean Data Analysis Society*, vol. 10, pp. 1339–1355, 2008.

[31] Y. Jang, Y. Yun, and J. Kwon, "The Study of the relation between the body mass index and the Dam-Bangkwang Body of Hyung-Sang medicine in patients over 45 years of age," *Journal of Korean Medicine*, vol. 36, no. 3, pp. 65–72, 2015.

[32] L. Zhao, S. Zhang, Z. Wang et al., "Efficacy of modified ban xia xie xin decoction on functional dyspepsia of cold and heat in complexity syndrome: a randomized controlled trial," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 812143, 8 pages, 2013.

[33] N. J. Talley, M. Haque, J. W. Wyeth et al., "Development of a new dyspepsia impact scale: the Nepean Dyspepsia Index," *Alimentary Pharmacology and Therapeutics*, vol. 13, no. 2, pp. 225–235, 1999.

[34] N. J. Talley, M. Verlinden, and M. Jones, "Quality of life in functional dyspepsia: responsiveness of the Nepean Dyspepsia Index and development of a new 10-item short form," *Alimentary Pharmacology and Therapeutics*, vol. 15, no. 2, pp. 207–216, 2001.

[35] S. Lee, S. Choi, Y. Cho, and M. Choi, "Validation of the Nepean Dyspepsia Index-Korean version," *Korean Journal of Neurogastroenterology and Motility*, vol. 14, p. 48, 2003.

[36] M. Lee, N. Y. Bae, M. Hwang, and H. Chae, "Development and validation of the digestive function assessment instrument for traditional Korean medicine: Sasang digestive function inventory," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 263752, 10 pages, 2013.

[37] S. Zhang, Z. Chen, and W. Xu, "Study on distribution characteristic of syndrome of 565 cases of functional dyspepsia by twice differentiation of symptoms and signs based on the 'cold, heat, deficiency, excess,'" *China Journal of Traditional Chinese Medicine and Pharmacy*, vol. 23, pp. 833–834, 2008.

[38] S. Zhang, H. Wang, and Q. Li, "Chinese consensus on diagnosis and treatment of functional dyspepsia," *Chinese Journal of Integrated Traditional and Western Medicine on Digestion*, vol. 30, pp. 533–537, 2010.

[39] H. Oh, J. Lee, J. Kim et al., "Study on the development of a standard instrument of diagnosis and assessment for spleen Qi deficiency pattern," *The Journal of Korean Oriental Medicine*, vol. 35, pp. 157–170, 2014.

[40] H. Oh, J. Lee, J. Kim, and J. Lee, "Exploratory study on the pre- and post-prandial subjective appetite and plasma gut hormone levels in spleen qi deficiency (SQD) syndrome," *Journal of Sasang Constitutional Medicine*, vol. 27, no. 1, pp. 125–137, 2015.

[41] D. D. Pham, J.-H. Do, B. Ku, H. J. Lee, H. Kim, and J. Y. Kim, "Body mass index and facial cues in Sasang typology for young and elderly persons," *Evidence-Based Complementary & Alternative Medicine*, vol. 2011, Article ID 749209, 9 pages, 2011.

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Research Article

Cold and Spleen-Qi Deficiency Patterns in Korean Medicine Are Associated with Low Resting Metabolic Rate

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Background. Korean medicine (KM) patterns such as cold, heat, deficiency, and excess patterns have been associated with alterations of resting metabolic rate (RMR). However, the association of KM patterns with accurately measured body metabolic rate has not been investigated. *Methods.* Data on cold (CP), heat (HP), spleen-qi deficiency (SQDP), and kidney deficiency (KDP) patterns were extracted by a factor analysis of symptoms experienced by 954 participants. A multiple regression analysis was conducted to determine the association between KM patterns and RMR measured by an indirect calorimeter. *Results.* The CP and SQDP scores were higher and the HP score was lower in women. The HP and SQDP scores decreased with age, while KDP scores increased with age. A multiple regression analysis revealed that CP and SQDP scores were negatively associated with RMR independently of gender and age, and the CP remained significantly and negatively associated with RMR even after adjustment for fat-free mass. *Conclusions.* The underlying pathology of CP and SQDP might be associated with the body's metabolic rate. Further studies are needed to investigate the usefulness of RMR measurement in pattern identification and the association of CP and SQDP with metabolic disorders.

1. Introduction

Resting metabolic rate (RMR) is the amount of energy needed by the body to maintain homeostatic functions during resting conditions. It constitutes the largest fraction of total energy expenditure, accounting for about 65–70% [1]. RMR can be determined using indirect calorimetry by measuring oxygen consumption and carbon dioxide production, which is considered to be the gold standard for assessing RMR [2]. It has been demonstrated that RMR is influenced by various factors, including gender, age, ethnicity, and body composition. Among those, fat-free mass (FFM) has been recognized to be the major determinant of RMR [3]. Alterations in RMR are associated with obesity, metabolic syndrome, diabetes mellitus, multimorbidity, and even mortality [4–8].

Pattern identification, also known as syndrome differentiation, is an essential part of diagnosis in Korean medicine (KM). During the process of pattern identification, all symptoms and signs are analyzed to determine the patient's physical condition and the cause, nature, and location of a disease [9]. Pattern identification is principally used to guide medical intervention, and some studies have claimed that it improves the successful treatment outcome rate when used in both Eastern and Western interventions [10, 11].

Various books and research papers on KM have described the underlying pathology of KM patterns in relation to decreased or increased metabolic rate. For example, a cold pattern and a deficiency pattern are often reported to be related to a lowered body metabolism, while a heat pattern and excess pattern are related to excessive hyperactivity of the body metabolism, suggesting that the symptoms of the KM patterns such as cold/hot sensation in the body, decreased/increased sweating, and clear/red color of urine may be related to the altered body metabolic rate [12–15].

Few studies, however, have investigated the association of KM patterns with accurately measured body metabolic rate. To our knowledge, there was only one study conducted with twelve Japanese women that reported that RMR was lower in women with cold pattern than in women without cold pattern [16]. Thus, this study aims to further investigate the association of KM patterns with RMR and anthropometric and body composition measurements in a larger sample.

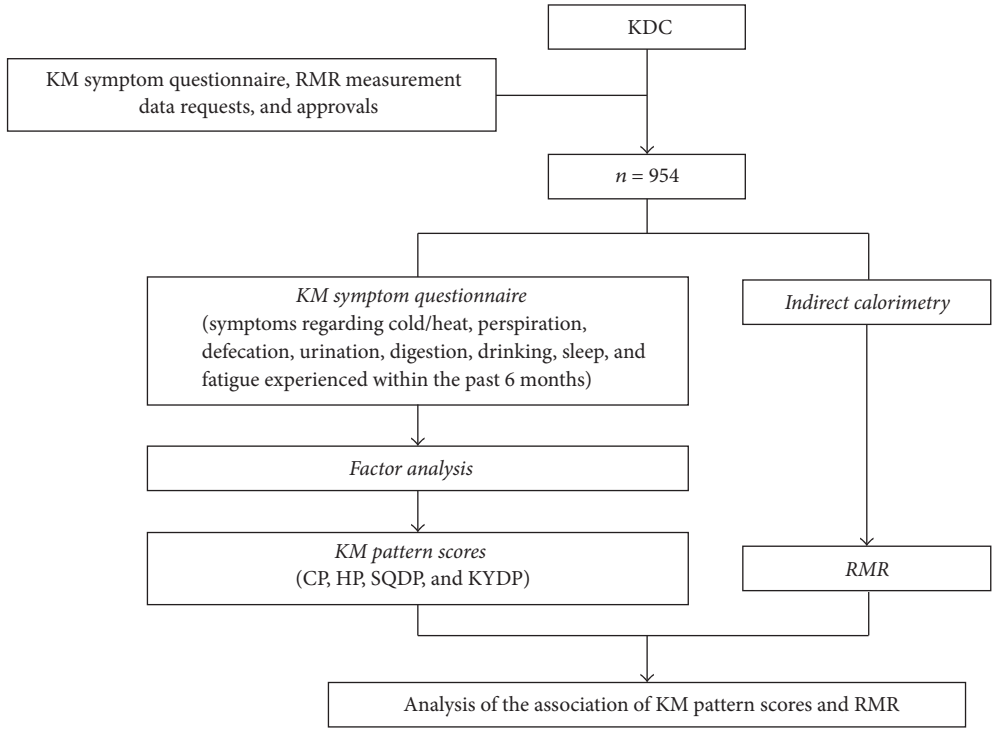

Figure 1: Flow chart of the study procedure. KDC, Korean Medicine Data Center; CP, cold pattern; SQDP, spleen-qi deficiency pattern; HP, heat pattern; KDP, kidney deficiency pattern.

2. Materials and Methods

2.1. Participants. This cross-sectional study was conducted between 2009 and 2015. The KM symptom questionnaire, RMR, and body composition measurement data were derived from the Korean Medicine Data Center (KDC) of the Korea Institute of Oriental Medicine [17] (Figure 1). A total of 954 healthy volunteers between 20 and 70 years of age without any chronic disease and history of hospitalization in the previous 5 years were included in the study. The study was approved by the Korea Institute of Oriental Medicine (Number I-1004/001-003) and informed consent was obtained from all participants prior to inclusion in the study.

2.2. Data Collection

2.2.1. KM Patterns. Participants were asked to complete the KM symptom questionnaire that consisted of questions about symptoms experienced by the individual within the past 6 months. The symptoms referred to certain conditions, that is, cold/heat, perspiration, defecation, urination, digestion, drinking, sleep, and fatigue, which are deemed important during the basic examination in KM [12]. A factor analysis was conducted to identify symptom patterns. Four patterns, namely, cold pattern (CP), heat pattern (HP), spleen-qi deficiency pattern (SQDP), and kidney deficiency pattern (KDP), were extracted along with their respective scores (Supplementary Table S1 in Supplementary Material available online at https://doi.org/10.1155/2017/9532073). CP was characterized by cold sensation in the feet, cold sensation in the hands, no reddish urine, cold sensation in the abdomen, preference for drinking warm water, and frequent urination; HP was characterized by no aversion to cold, increased sweating, and no preference for drinking warm water; SQDP was characterized by irregular defecation, less frequent bowel movements, feeling of incomplete defecation, indigestion, and fatigue; and KDP was characterized by urination at night, awakening during the night, poor quality of sleep, frequent urination, and loose/watery stool (Supplementary Table S2).

2.2.2. RMR Measurements. Participants were asked to fast and refrain from stimulants (smoking, alcohol, and coffee) and heavy exercise for at least 12 h prior to visiting the laboratory. RMR was measured with a breath-by-breath gas exchange analysis using an indirect calorimeter (Vmax ENCORE 29c; Sensormedic, Viasys HealthCare, Yorba Linda, CA, USA). The gas flow calibration as well as oxygen and carbon dioxide analyzer calibration was performed according to the manufacturer's instructions. RMR was measured for 20 min while participants were awake and lying in a supine position. Oxygen uptake and carbon dioxide production were measured and RMR was calculated using the Weir equation [18].

2.2.3. Body Composition Measurements. Body height was measured with a digital scale (GL-150; G Tech International Co., Uijeongbu, Korea), and weight, fat-free mass (FFM), and body fat mass (BFM) were measured with a bioelectrical impedance analyzer (Inbody 720, InBody, Seoul, South

Table 1: Characteristics of the study participants.

	All (n = 954)	Men (n = 454)	Women (n = 500)	p value
Height (cm)	166.0 ± 8.6	173.0 ± 5.8	159.7 ± 5.3	<0.001
Weight (kg)	64.0 ± 11.5	71.9 ± 10.0	56.9 ± 7.4	<0.001
BMI (kg/m^2)	23.1 ± 3.0	24.0 ± 2.9	22.3 ± 2.9	<0.001
FFM (kg)	47.8 ± 9.9	56.7 ± 6.2	39.7 ± 3.9	<0.001
BFM (kg)	16.2 ± 5.7	15.2 ± 5.9	17.2 ± 5.3	<0.001
RMR (kcal/day)	1378.9 ± 313.5	1579.5 ± 293.1	1197.2 ± 200.2	<0.001

BMI, body mass index; FFM, fat-free mass; BFM, body fat mass; RMR, resting metabolic rate.

Table 2: Correlation coefficients of KM pattern scores with anthropometric and body composition indices.

	Unadjusted				Adjusted			
	CP	SQDP	HP	KDP	CP	SQDP	HP	KDP
Height	**−0.291****	**−0.070***	**0.137****	**−0.112****	0.035	−0.005	−0.005	−0.006
Weight	**−0.439****	**−0.147****	**0.207****	−0.038	**−0.239****	**−0.086****	**0.168****	0.009
BMI	**−0.373****	**−0.140****	**0.171****	0.050	**−0.284****	**−0.083***	**0.186****	0.023
FFM	**−0.432****	**−0.148****	**0.158****	**−0.100****	**−0.168****	**−0.100****	**0.065***	−0.037
BFM	**−0.134****	−0.042	**0.142****	**0.099****	**−0.224****	−0.044	**0.206****	0.048

Unadjusted, Pearson's correlation coefficients; adjusted, partial correlation coefficients adjusted for gender and age; CP, cold pattern; SQDP, spleen-qi deficiency pattern; HP, heat pattern; KDP, kidney deficiency pattern; $^{*}p < 0.05$; $^{**}p < 0.01$.

Korea). Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m).

2.3. Statistical Analysis. Sample characteristics were presented as the mean and standard deviation, and differences of sample characteristics and pattern scores between genders were compared using an independent t-test. The effect of age on pattern scores was analyzed by a simple regression within each gender. To examine the relationship of the pattern scores and anthropometric and body composition measurements, Pearson's correlation coefficients and partial correlation coefficients controlling for age and gender were estimated. To determine the association between pattern scores and RMR, a multiple regression analysis was conducted; this involved adjustment for age, gender, and FFM, which are known to be an influential factor of RMR [19]. A p value of less than 0.05 was considered statistically significant. Statistical analyses were performed using SPSS 22.0 (IBM, Chicago, IL, USA) and R (the R Foundation for Statistical Computing, Version 3.2.4).

3. Results

3.1. Characteristics of Participants. The characteristics of the study participants are shown in Table 1. Of the 954 participants, 500 (52.4%) were women and 454 (47.6%) were men. Their mean age was 37.8 ± 11.7 years. All anthropometric and body composition indices were statistically different between genders ($p < 0.01$). The mean RMR was higher in men (1579.5 ± 293.1 kcal/day) than in women (1197.2 ± 200.2 kcal/day) ($p < 0.01$).

3.2. Distribution of KM Pattern Scores by Gender and Age. The CP and SQDP scores were significantly higher ($p < 0.01$), while HP score was lower ($p < 0.01$) in women than in men. The CP score in women decreased with age ($p < 0.05$), which was not significant in men ($p > 0.05$). The SQDP and HP scores significantly decreased with age, while the KDP score increased with age in both genders ($p < 0.05$) (Figure 2).

3.3. Correlation of KM Pattern Scores with Anthropometric and Body Composition Indices. In correlation analysis with anthropometric and body composition indices, the CP and SQDP score showed negative correlations with height, weight, BMI, and FFM, while HP showed positive correlations with those indices. When adjusted for gender and age, the correlations of the CP, SQDP, and HP scores with anthropometric and body composition indices became weak but correlations with weight, BMI, and FFM remained significant. The KDP score has no significant correlation with any of the indices (Table 2).

3.4. Association of KM Pattern Scores with RMR. In Model 1, each of the KM pattern scores was entered into the regression model as an independent variable with RMR as a dependent variable. The CP and SQDP scores were negatively associated with RMR, while the HP score was positively associated with RMR. When gender and age were adjusted in Model 2, the CP and SQDP scores were significantly negatively associated with RMR. When additional adjustment of FFM was applied in Model 3, only the CP score was significantly associated with RMR (Table 3).

4. Discussion

The present study aimed to investigate the association between KM patterns and RMR. The pattern scores of CP, SQDP, HP, and KDP were different according to gender and age. In addition, CP, SQDP, and HP scores showed significant

Table 3: Multiple regression analysis for the association of KM pattern scores and RMR.

	Model 1			Model 2			Model 3		
	B	SE	*p* value	*B*	SE	*p* value	*B*	SE	*p* value
CP	−89.9	7.8	**<0.001**	−33.4	7.2	**<0.001**	−15.6	6.5	**0.016**
SQDP	−18.5	7.8	**0.018**	−16.4	6.8	**0.016**	−9.4	6.0	0.119
HP	36.1	7.8	**<0.001**	12.2	6.8	0.075	3.5	6.0	0.564
KDP	−7.4	7.9	0.345	7.6	7.2	0.291	10.9	6.4	0.087

Model 1: unadjusted; Model 2: adjusted for gender and age; Model 3: adjusted for gender, age, and fat-free mass; *B*, unstandardized coefficients; SE, standard error of unstandardized coefficients; RMR, resting metabolic rate; CP, cold pattern; SQDP, spleen-qi deficiency pattern; HP, heat pattern; KDP, kidney deficiency pattern.

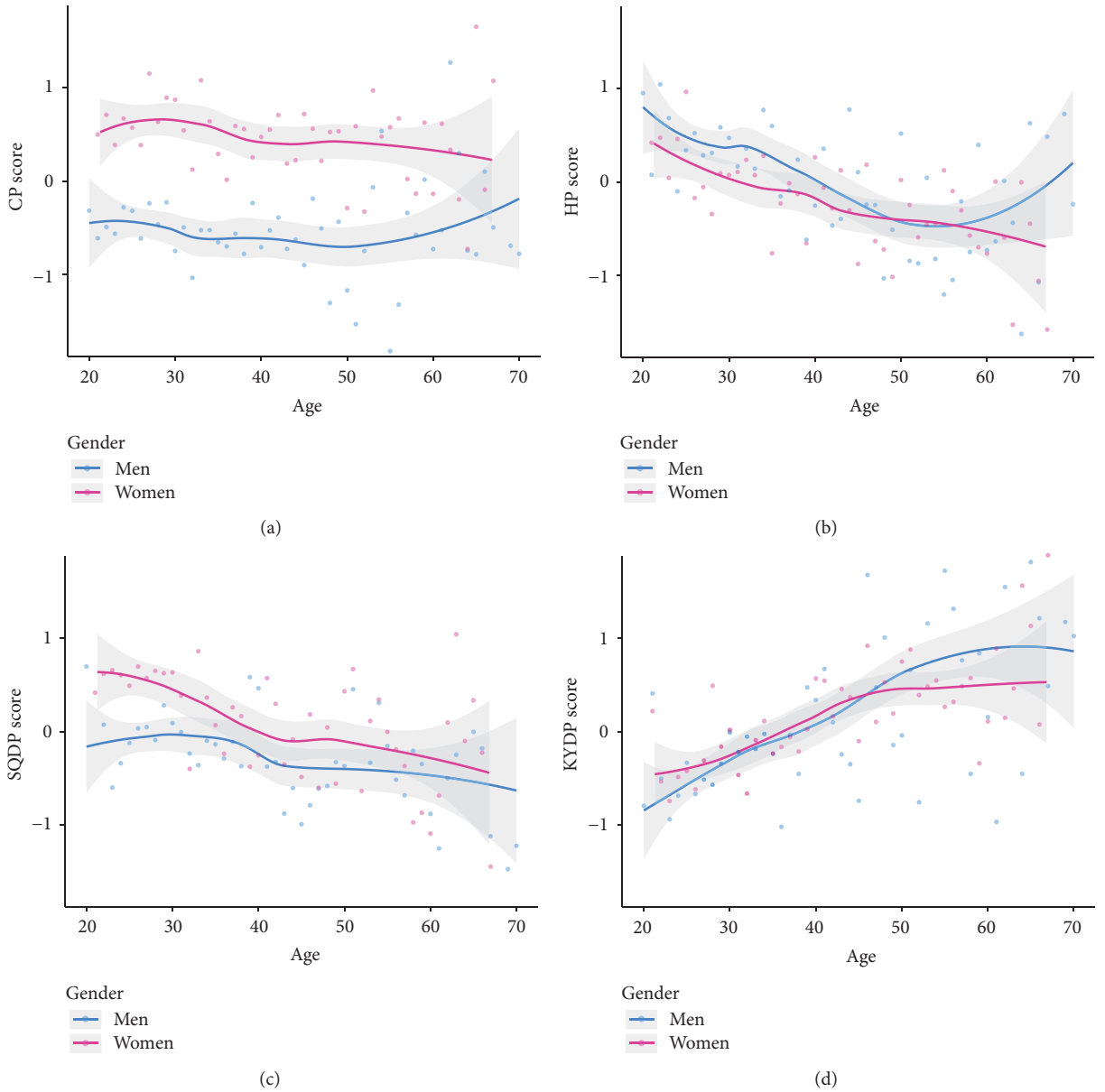

Figure 2: KM pattern scores by gender and age. Depicted are LOWESS smoothed lines and 95% confidence intervals (grey shade) with the observed means of pattern scores (dots) by gender and age. CP, cold pattern; SQDP, spleen-qi deficiency pattern; HP, heat pattern; KDP, kidney deficiency pattern.

correlations with anthropometric and body composition indices. A multiple regression analysis revealed that CP and SQDP scores were negatively associated with RMR independently of gender and age, suggesting an association of the underlying pathology of CP and SQDP with altered RMR.

CP is one of the eight basic principles of pattern identification and believed to indicate the nature of imbalance in the body. CP is more common in women than in men [13, 20], and the same trend was observed in our results. The representative symptoms of CP, such as a cold sensation in the extremities and aversion to cold, are also more common in women [21–23]. This seems natural based on the report that the cutaneous hand blood flow of women at room temperature is lower than in men [24]. Also, when exposed to cold stimuli, the cutaneous blood flow is reduced more largely in women than in men [25].

According to the KM theory, CP encompasses symptoms of a cold sensation in the body, aversion to cold, lack of thirst, pale facial color, clear urine, and a desire to lie down; thus, its underlying pathology is often associated with decreased metabolism of the whole body [12–15]. A previous study reported that RMR is lower in women with CP than in women without CP; however, it included a very small sample size of twelve [16]. Our study included 954 participants, which is a comparatively larger sample than that of other studies using indirect calorimetry, to accurately estimate RMR. Our results were consistent with those of a previous study showing that the RMR was negatively associated with the CP score; this association remained significant after adjusting for gender, age, and even FFM, which is known to be the most influential determinant of RMR.

One of the possible explanations for the low RMR in individuals with CP could be their low thyroid function [16, 26], because altered level of thyroid hormone, even at subclinical level, has been known to be responsible for changes of thermogenesis, affecting energy expenditure and RMR [27]. Hypothyroidism could cause increased sensitivity to cold, which is a major symptom of CP. Indeed, hypothyroidism has been reported to be significantly more common in individuals diagnosed as CP than normal controls [13]. On the other hand, it has been also reported that differences of RMR, even when normalized by FFM, could be caused by differences in the composition of FFM, which can be accurately measured by using multiscan computerized axial tomography or magnetic resonance imaging [1, 28]. This is because FFM constitutes various kinds of tissues and organs that have different metabolic rates. Because our results indicate that the CP was significantly associated with RMR independently of the total FFM, the different composition of FFM in individuals with CP could be one of the explanations for the association between RMR and CP.

Several studies that investigated the different treatment effects in patients with rheumatoid arthritis found that effects of traditional East Asian medicine or even Western medicine could differ among patients with different characteristics related to the CP [11, 29]. This indicates that people with or without CP have different pathophysiological conditions that affect the treatment outcome. Because our results showed that RMR was significantly associated with CP, we could assume that one of the different pathological conditions between people with and without CP, which lead to different treatment effect, could be their different rate of body metabolism.

SQDP is mainly characterized by gastrointestinal problems, such as irregular defecation, reduced bowel movement, indigestion, and fatigue. This pattern is defined as a pathological change characterized by qi deficiency with impaired transporting and transforming function of the spleen [9]. Gastrointestinal problems could lead to altered eating habits that might reduce the total energy intake [30, 31]. RMR is known to decrease in response to restriction of energy intake to values below predictions based on body composition changes, possibly due to an adaptation which can limit weight loss and compromise the maintenance of a reduced body weight [32]. This mechanism could be one of the explanations for the low RMR in individuals with SQDP.

Our results suggest that CP and SQDP were significantly associated with RMR after adjustment for gender and age. This means that RMR is different according to the status of CP and SQDP in individuals with the same gender and age. Even though FFM is known to be the most influential determinant of RMR, FFM should be measured with a device such as a bioelectrical impedance analyzer or a dual-energy X-ray absorptiometer. Accordingly, the results indicate that evaluating CP and SQDP could be helpful to predict RMR of patients in a more practical way in clinical settings. Moreover, the CP was negatively associated with RMR after additional adjustment for FFM. Thus, consideration of the CP is recommended even when estimating an individual's RMR based on his/her FFM value.

The KM diagnostic process has been criticized for its subjectivity and dependence on the doctor's experience; thus the need to establish standardized diagnostic method using objective and quantitative methods has been asserted in the literature [33, 34]. Because our results showed the significant association of the CP and SQDP with RMR, we could assume that we may identify patients with CP and SQDP not only with traditionally used diagnostic method of questioning patients about symptoms and checking clinical signs such as tongue appearance and pulse evaluated by KM doctors, but also with objective and quantitative method of measuring RMR of patients. Also, our results suggest that reduced RMR could be one of the underlying pathologies of the relevant patterns, which means it is possible that the treatments which have been used for patients with the CP and SQDP are effective by means of directly or indirectly controlling the altered RMR of patients. Thus, the usefulness of measuring RMR in clinical practice for pattern identification and evaluation of clinical process should be further investigated in future studies.

Our results showed that the HP score was higher in men and showed positive associations with weight, BMI, FFM, and BFM and negative association with RMR, which was the opposite of CP. However, unlike the CP, the association of the HP with RMR was not significant after adjusting for gender and age. This is possibly due to the different composition of the symptom characteristics that are related to HP and CP in our study. The CP in our study was extracted by a factor analysis and mostly constituted symptoms related to thermal

sensation of the body, while the HP mostly constituted symptoms related to preferred ambient temperature or sweating amount. However, according to KM theory, CP and HP are basically two patterns that reflect the nature of imbalance in the body and indicate the relative exuberance and debility of the yin and yang of the body. Consequently, the manifestations are conceptually contrast to each other; for example, the CP includes the symptoms of aversion to cold, preference for warmth, and a pale face, while the HP includes the symptoms of aversion to heat, preference for coolness, and a red face. Based on our results, it seems that the symptoms that are known to be relevant to the CP and HP are not necessarily analyzed as just one pattern. Our results could be interpreted as that the symptom pattern that was mostly about thermal sensation in the direction of cold was significantly associated with RMR and the symptom pattern that was mostly about preference for ambient temperature and sweating amount in the direction of heat were not significantly associated with RMR.

KDP is characterized by urination at night and sleep problems of awakening during the night and poor quality of sleep. The KDP score showed a significant increase with age, which is consistent with the KM theory that kidney function deteriorates with age [35]. The KDP score tended to decrease with increased RMR; however, this was not statistically significant.

Although there were studies that utilized a factor analysis of symptoms to statistically identify KM patterns [11, 29, 36], to the best of our knowledge, this is the first study to investigate the association of RMR with KM patterns based on a factor analysis. In pattern identification, symptoms have been utilized as key factors and the existence of a symptom pattern is screened rather than the existence of a single symptom. Predefined questionnaires for a specific pattern, which only include symptoms confined to that specific pattern, have been widely used in KM research, usually accompanying pattern scores of summing the number of pertinent symptoms [37–39]. However, in those studies, the statistically meaningful tendency of cooccurrence of symptoms could not be verified. In our study, a wide range of symptoms that are screened in basic examination in KM and possibly related to multiple KM patterns were analyzed by factor analysis, which yielded similar symptom patterns to CP, HP, SQDP, and KDP.

Some caution is needed when interpreting the results of this study. Firstly, data of healthy participants were analyzed in the study instead of patients with a specific disease. We expect symptoms related to KM patterns to be more prevalent and severe in patients with diseases and future studies to investigate the associations of KM patterns with RMR in various disease groups are required. Secondly, symptoms used in this study were self-reported, based on recall over the previous 6 months. Although self-administered questionnaires on symptoms have been widely used to evaluate an individual's health [40–42], this may be prone to a recall bias. We recommend that major clinical signs, such as the tongue appearance and pulse examination, should be included in future studies, to investigate their associations with symptom patterns and RMR.

5. Conclusions

Our results showed that the individuals with higher CP and SQDP scores have lower RMR when compared to individuals of the same gender and age with lower CP and SQDP scores, suggesting that the underlying pathology of CP and SQDP may be associated with the body's metabolic rate. In consideration of the effect of altered RMR on metabolic disorders, evaluating the CP and SQDP in patients with metabolic disorders is recommended. In addition, to complement the subjectivity of the current KM diagnosis process, further studies are required to investigate the usefulness of RMR measurement in CP and SQDP pattern identification.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contributions

Sujeong Mun analyzed the data and drafted the manuscript. Sujung Kim provided statistical advice and helped in the interpretation of the data. Kwang-Ho Bae helped in the interpretation of the data and revising the manuscript. Siwoo Lee conceived of the study and participated in its design and coordination. All of the authors read and approved the final manuscript.

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References

[1] Z. Wang, S. Heshka, K. Zhang, C. N. Boozer, and S. B. Heymsfield, "Resting energy expenditure: systematic organization and critique of prediction methods," *Obesity Research*, vol. 9, no. 5, pp. 331–336, 2001.

[2] P. J. M. Weijs, "Validity of predictive equations for resting energy expenditure in US and Dutch overweight and obese class I and II adults aged 18–65 y," *American Journal of Clinical Nutrition*, vol. 88, no. 4, pp. 959–970, 2008.

[3] S. B. Heymsfield, D. Gallagher, D. P. Kotler, Z. Wang, D. B. Allison, and S. Heshka, "Body-size dependence of resting energy expenditure can be attributed to nonenergetic homogeneity of fat-free mass," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 282, no. 1, pp. E132–E138, 2002.

[4] S. Buscemi, S. Verga, G. Caimi, and G. Cerasola, "A low resting metabolic rate is associated with metabolic syndrome," *Clinical Nutrition*, vol. 26, no. 6, pp. 806–809, 2007.

[5] K. Martin, P. Wallace, P. F. Rust, and W. T. Garvey, "Estimation of resting energy expenditure considering effects of race and diabetes status," *Diabetes Care*, vol. 27, no. 6, pp. 1405–1411, 2004.

[6] M. Monda, G. Messina, C. Mangoni, and B. De Luca, "Resting energy expenditure and fat-free mass do not decline during aging in severely obese women," *Clinical Nutrition*, vol. 27, no. 4, pp. 657–659, 2008.

[7] E. Fabbri, Y. An, J. A. Schrack et al., "Energy metabolism and the burden of multimorbidity in older adults: results from the Baltimore longitudinal study of aging," *Journals of Gerontology—Series A Biological Sciences and Medical Sciences*, vol. 70, no. 11, pp. 1297–1303, 2015.

[8] C. Ruggiero, E. J. Metter, V. Melenovsky et al., "High basal metabolic rate is a risk factor for mortality: the Baltimore Longitudinal Study of Aging," *Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, vol. 63, no. 7, pp. 698–706, 2008.

[9] WHO Regional Office for the Western Pacific, *WHO International Standard Terminologies on Traditional Medicine in the Western Pacific Region*, World Health Organization, Geneva, Switzerland, 2007.

[10] A. S. Ferreira and A. J. Lopes, "Chinese medicine pattern differentiation and its implications for clinical practice," *Chinese Journal of Integrative Medicine*, vol. 17, no. 11, pp. 818–823, 2011.

[11] C. Lu, Q. Zha, A. Chang, Y. He, and A. Lu, "Pattern differentiation in traditional chinese medicine can help define specific indications for biomedical therapy in the treatment of rheumatoid arthritis," *Journal of Alternative and Complementary Medicine*, vol. 15, no. 9, pp. 1021–1025, 2009.

[12] T. Deng, *Practical Diagnosis in Traditional Chinese Medicine*, Churchill Livingstone, Edinburgh, UK, 1999.

[13] T. Yoshino, K. Katayama, K. Munakata et al., "Statistical analysis of hie (cold sensation) and hiesho (cold disorder) in Kampo clinic," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 398458, 8 pages, 2013.

[14] Z. Xu, Q. Chen, Q. Sun et al., "Heat syndrome of traditional Chinese medicine," *Chinese Journal of Comparative Medicine*, vol. 19, no. 3, pp. 53–56, 2009.

[15] Y.-T. Yin, X.-W. Li, Y. Dong, and J.-R. Shi, "Research progress in biological basis of cold and heat essence of Chinese medicine," *Zhong Xi Yi Jie He Xue Bao*, vol. 10, no. 12, pp. 1328–1335, 2012.

[16] K. Nagashima, T. Yoda, T. Yagishita, A. Taniguchi, T. Hosono, and K. Kanosue, "Thermal regulation and comfort during a mild-cold exposure in young Japanese women complaining of unusual coldness," *Journal of Applied Physiology*, vol. 92, no. 3, pp. 1029–1035, 2002.

[17] H.-J. Jin, Y. Baek, H.-S. Kim, J. Ryu, and S. Lee, "Constitutional multicenter bank linked to Sasang constitutional phenotypic data," *BMC Complementary and Alternative Medicine*, vol. 15, no. 1, article 46, 2015.

[18] J. B. D. V. Weir, "New methods for calculating metabolic rate with special reference to protein metabolism," *The Journal of Physiology*, vol. 109, no. 1-2, pp. 1–9, 1949.

[19] T. T. Haaf and P. J. M. Weijs, "Resting energy expenditure prediction in recreational athletes of 18-35 years: confirmation of cunningham equation and an improved weight-based alternative," *PLOS ONE*, vol. 9, no. 10, Article ID 0108460, 2014.

[20] T. Baek, Y.-J. Park, Y.-B. Park, J.-H. Park, and J.-J. Im, "Health test for searching of correlation and the index of the cold-heat patternization comparison of the questionnaires for the cold-heat patternization and the ordinary," *The Journal of the Korea Institute of Oriental Medical Diagnostics*, vol. 9, no. 2, pp. 145–151, 2005.

[21] S. Karjalainen, "Thermal comfort and gender: a literature review," *Indoor Air*, vol. 22, no. 2, pp. 96–109, 2012.

[22] A. Yasuoka, H. Kubo, K. Tsuzuki, and N. Isoda, "Interindividual differences in thermal comfort and the responses to skin cooling in young women," *Journal of Thermal Biology*, vol. 37, no. 1, pp. 65–71, 2012.

[23] M. Mozaffarieh, P. Fontana Gasio, A. Schötzau, S. Orgül, J. Flammer, and K. Kräuchi, "Thermal discomfort with cold extremities in relation to age, gender, and body mass index in a random sample of a Swiss urban population," *Population Health Metrics*, vol. 8, no. 1, article no. 17, 2010.

[24] K. Cankar, Ž. Finderle, and M. Štrucl, "Gender differences in cutaneous laser doppler flow response to local direct and contralateral cooling," *Journal of Vascular Research*, vol. 37, no. 3, pp. 183–188, 2000.

[25] R. Solianik, A. Skurvydas, A. Vitkauskiene, and M. Brazaitis, "Gender-specific cold responses induce a similar body-cooling rate but different neuroendocrine and immune responses," *Cryobiology*, vol. 69, no. 1, pp. 26–33, 2014.

[26] M. Lee and Y. Choi, "Systemic reviews of domestic experimental studies of herbal medicines used for hypothyroidism since 2000," *The Journal of Internal Korean Medicine*, vol. 36, no. 4, pp. 570–581, 2015.

[27] T. Hagedorn, E. Poggiogalle, C. Savina et al., "Indirect calorimetry in obese female subjects: factors influencing the resting metabolic rate," *World Journal of Experimental Medicine*, vol. 2, no. 3, pp. 58–64, 2012.

[28] M. J. Müller, A. Bosy-Westphal, D. Kutzner, and M. Heller, "Metabolically active components of fat-free mass and resting energy expenditure in humans: recent lessons from imaging technologies," *Obesity Reviews*, vol. 3, no. 2, pp. 113–122, 2002.

[29] Y. He, A. Lu, C. Lu et al., "Symptom combinations assessed in traditional Chinese medicine and its predictive role in ACR20 efficacy response in rheumatoid arthritis," *American Journal of Chinese Medicine*, vol. 36, no. 4, pp. 675–683, 2008.

[30] B. F. Filipović, T. Randjelovic, N. Kovacevic et al., "Laboratory parameters and nutritional status in patients with functional dyspepsia," *European Journal of Internal Medicine*, vol. 22, no. 3, pp. 300–304, 2011.

[31] P. G. L. Speridião, S. Tahan, U. Fagundes-Neto, and M. B. Morais, "Dietary fiber, energy intake and nutritional status during the treatment of children with chronic constipation," *Brazilian Journal of Medical and Biological Research*, vol. 36, no. 6, pp. 753–759, 2003.

[32] S. Camps, *How Humans Economize: Energy Restriction and End Energy Expenditure*, Uitgeverij BOXPress, 2015.

[33] J. A. Lee, T.-Y. Park, J. Lee et al., "Developing indicators of pattern identification in patients with stroke using traditional Korean medicine," *BMC Research Notes*, vol. 5, article no. 136, 2012.

[34] M. Jiang, C. Lu, C. Zhang et al., "Syndrome differentiation in modern research of traditional Chinese medicine," *Journal of Ethnopharmacology*, vol. 140, no. 3, pp. 634–642, 2012.

[35] H. Xu, D. Lawson, A. Kras, and D. Ryan, "The use of preventive strategies for bone loss," *American Journal of Chinese Medicine*, vol. 33, no. 2, pp. 299–306, 2005.

[36] V. Scheid, T. Ward, and V. Tuffrey, "Comparing TCM textbook descriptions of menopausal syndrome with the lived experience of London women at midlife and the implications for Chinese medicine research," *Maturitas*, vol. 66, no. 4, pp. 408–416, 2010.

[37] B.-K. Kang, T.-Y. Park, J. A. Lee et al., "Reliability and validity of the Korean standard pattern identification for stroke (K-SPI-Stroke) questionnaire," *BMC Complementary and Alternative Medicine*, vol. 12, article no. 55, 2012.

[38] H. Ryu, H. Lee, H. Kim, and J. Kim, "Reliability and validity of a cold-heat pattern questionnaire for traditional Chinese medicine," *Journal of Alternative and Complementary Medicine*, vol. 16, no. 6, pp. 663–667, 2010.

[39] C. A. Berle, D. Cobbin, N. Smith, and C. Zaslawski, "A novel approach to evaluate traditional Chinese medicine treatment outcomes using pattern identification," *Journal of Alternative and Complementary Medicine*, vol. 16, no. 4, pp. 357–367, 2010.

[40] L. Wang, X. Zhao, X. Wu et al., "Diagnosis analysis of 4 TCM patterns in suboptimal health status: a structural equation modelling approach," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 970985, 6 pages, 2012.

[41] X. Zhou, F. Xu, J. Gao et al., "Development and preliminary validation of the questionnaire (the First Edition) based on TCM for detecting health status in China," *Evidence-based Complementary and Alternative Medicine*, vol. 2015, Article ID 863685, 12 pages, 2015.

[42] H. Zhao, W.-H. Xiong, X. Zhao, L.-M. Wang, and J.-X. Chen, "Development and evaluation of a Traditional Chinese Medicine syndrome questionnaire for measuring sub-optimal health status in China," *Journal of Traditional Chinese Medicine*, vol. 32, no. 2, pp. 129–136, 2012.