Effects of manual therapy combined with therapeutic exercise versus routine physical therapy on brain biomarkers in patients with chronic non-specific neck pain in Thailand: a study protocol for a single-blinded randomised controlled trial

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ABSTRACT

Introduction Structural brain alterations in pain-related areas have been demonstrated in patients with non-specific neck pain. While manual therapy combined with therapeutic exercise is an effective management for neck pain, its underlying mechanisms are poorly understood. The primary objective of this trial is to investigate the effects of manual therapy combined with therapeutic exercise on grey matter volume and thickness in patients with chronic non-specific neck pain. The secondary objectives are to assess changes in white matter integrity, neurochemical biomarkers, clinical features of neck pain, cervical range of motion and cervical muscle strength.

Methods and analysis This study is a single-blinded, randomised controlled trial. Fifty-two participants with chronic non-specific neck pain will be recruited into the study. Participants will be randomly allocated to either an intervention or control group (1:1 ratio). Participants in the intervention group will receive manual therapy combined with therapeutic exercise for 10 weeks (two visits per week). The control group will receive routine physical therapy. Primary outcomes are whole-brain and regional grey matter volume and thickness. Secondary outcomes are white matter integrity (fractional anisotropy and mean diffusivity), neurochemical biomarkers (N-acetylaspartate, creatine, glutamate/glutamine, myoinositol and choline), clinical features (neck pain intensity, duration, neck disability and psychological symptoms), cervical range of motion and cervical muscle strength. All outcome measures will be taken at baseline and postintervention.

Ethics and dissemination Ethical approval of this study has been granted by Faculty of Associated Medical Science, Chiang Mai University. The results of this trial will be disseminated through a peer-reviewed publication.

Trial registration number NCT05568394.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This trial is a single-blinded randomised controlled trial to evaluate the effects of manual therapy combined with therapeutic exercise on structural brain and neurochemical biomarkers in chronic non-specific neck pain.
⇒ Both grey matter and white matter in the brain are assessed in the trial.
⇒ Assessments are measured only at baseline and postintervention.
⇒ It is not possible to blind the physiotherapists or study participants.

INTRODUCTION

It has been suggested that chronic pain is associated with maladaptive plasticity of nociceptive pathways through central sensitisation and remodelling of brain connectivity.1 Central sensitisation is mediated by increased neuronal activity, potentiated synaptic efficacy, enlarged receptive fields and reduced inhibition. Altered synaptic plasticity (connectivity) is involved in functional and structural remodelling in the brain. Most previous studies demonstrated decreased grey matter volume of whole-brain and specific brain regions (ie, the cingulate cortex, prefrontal cortex (PFC), thalamus, insula, precentral cortex, temporal cortex and precuneus) in various chronic musculoskeletal conditions.2–5 Decreased fractional anisotropy (FA) and white matter volume in specific brain regions (ie, the corpus callosum and internal capsule) were also demonstrated.5 The exact process underlying the structural changes...
remains unclear, however, the observed brain alterations are proposed as a consequence of chronic pain, which pain network modulation seems to aim at reinstating equilibrium between nociceptive and antinociceptive modulation.⁷

In chronic neck pain, altered brain structures have been observed both non-specific origin and whiplash-associated disorder (WAD).²³⁸ de Zoete et al.²⁰ identified decreased cortical volumes in several brain areas (ie, precentral, frontal, occipital, parietal, temporal and paracentral cortices) in patients with chronic non-specific neck pain and found moderate to strong correlations between structural brain morphology (volume and thickness) and clinical features of neck pain (duration, intensity and disability) and depression. De Pauw et al.⁶ revealed cortical thickening in the precuneus and superior parietal gyrus and increased grey matter volume in the superior parietal gyrus in patients with chronic non-specific neck pain and decreased grey matter volume in the precentral and superior temporal gyrus in patients with WAD. Changes in brain volume were also associated with pain intensity, neuromuscular control and cervical muscle strength. In addition, Coppieters et al.⁹ found abnormalities in white matter tracts carrying information between regions involved in affective-cognitive dimensions of pain processing and cognition (ie, cortical thinning in the precuneus, decreased FA in the cingulum hippocampus and tapetum) in women with chronic WAD compared with those with non-specific neck pain and controls.

Structural brain alterations may be useful biomarkers to evaluate clinical improvement in chronic pain. There is evidence suggesting that brain structures/neurobiology change in response to clinical intervention in patients with chronic pain.⁶–¹⁵ Physical exercise has been shown to induce activity in several brain regions (eg, PFC, insula, cerebellum and thalamus) in patients with chronic musculoskeletal conditions.¹¹ Spinal manipulation therapy has an effect on the sensory, emotion and cognition-associated areas (ie, precuneus, postcentral gyrus, posterior cingulate cortex and the superior frontal gyrus) of patients with chronic low back pain.¹² In chronic neck pain, manual therapy combined with therapeutic exercise is effective in improving pain, disability, cervical mobility and function and quality of life.¹⁴¹⁵ It has also been suggested that manual therapy and therapeutic exercise involve neurophysiological mechanisms.¹⁶¹⁷ However, the underlying biomarkers of these in chronic neck pain remain unclear. In addition, neurochemical changes in the brain (eg, N-acetyl aspartate, N-acetylaspartate, NAA, choline, Cho, glutamate/glutamine, Glu/Gln) may potentially be associated with the pathophysiology of chronic pain.¹⁸–²⁰ A few studies revealed unchanged neurochemical levels in patients with WAD.²¹²² Yet, there is no evidence to support neurochemical changes in patients with non-specific neck pain. A positron emission tomography study found brain metabolic changes (ie, increased glucose metabolism in the anterior cingulate cortex and cerebellar vermis and decreased in PFC) after spinal manipulation therapy in patients with non-specific neck pain.²³ Thus, neurochemical changes may advance understanding of the underlying mechanisms associated with manual therapy combined with therapeutic exercise in chronic non-specific neck pain.

The primary aim of the trial is to investigate the effects of manual therapy combined with therapeutic exercise on the brain’s grey matter compared with routine physical therapy treatment in patients with chronic non-specific neck pain, which is the most common form of neck pain.²⁴ The secondary aims are to investigate the effects of manual therapy combined with therapeutic exercise on white matter integrity, neurochemical biomarkers, clinical features (neck pain intensity and neck disability), psychological symptoms, cervical range of motion (CROM) and cervical muscle strength.

**HYPOTHESES**

Primary hypothesis: Based on the finding of previous studies,⁶–¹⁵ we expect that manual therapy combined with therapeutic exercise will be superior to routine physical therapy, in improving whole-brain grey matter volume, using surface-based morphometry analyses. In addition, we further expect changes in grey matter volume and cortical thickness in some brain regions related to pain (ie, primary motor cortex (M1), insula, PFC, cingulate cortex, precuneus, periaqueductal grey matter, primary somatosensory cortex (S1) and/or thalamus) after the interventions.

Secondary hypothesis: Manual therapy combined with therapeutic exercise will be superior to routine physical therapy in improving whole-brain white matter integrity (ie, FA and mean diffusivity, MD) using tract-based spatial statistics (TBSS), neurochemical biomarkers, neck pain intensity, neck disability, psychological symptoms, CROM and cervical muscle strength.

**METHODS AND ANALYSIS**

**Patient and public involvement**

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Study design and setting**

This study is a single-blinded, randomised controlled trial. Participants will be randomly allocated to either an intervention or control group. This trial conforms to the CONSORT (Consolidated Standards of Reporting Trials) recommendations and is registered on ClinicalTrials.gov (NCT05568394). The trial will be conducted at the research unit at the Department of Physical Therapy and the Department of Radiologic Technology, Chiang Mai University.

**Participants**

A sample of 52 people with chronic non-specific neck pain will be recruited into the study from hospitals, physical
therapy clinics, community, university and social media (eg, Facebook). Those who are interested in participating in the study will be screened by a research assistant via telephone interview. They will be eligible for the trial if they meet study eligibility criteria.

Inclusion criteria: aged between 18 and 59 years; a specific neck pain for ≥3 months; and an average pain intensity over the past week ≥35 mm on a Visual Analogue Scale (VAS).26

Exclusion criteria: a history of head and neck injury or surgery; known or suspected vestibular pathology or dizziness caused by underlying pathology in the ear, brain and sensory nerve pathways (eg, benign paroxysmal positional vertigo) and/or vascular disorders; neurological or musculoskeletal conditions that could affect the outcomes (eg, scoliosis, torticollis, myofascial pain syndrome, fibromyalgia and rheumatoid arthritis); metabolic conditions (eg, diabetes, obesity (body mass index >30 kg/m²) and hypertension); psychological symptoms (eg, anxiety, depression and schizophrenia); contraindications to MRI (eg, pregnancy/breast feeding, claustrophobia and ferromagnetic implants); and receiving physiotherapy treatment for their neck conditions in the past 12 months.

Procedure
The flow chart of the trial is shown in figure 1. Participant recruitment will start in November 2022 and is expected to complete in December 2023. Eligible participants will be randomly allocated to one of two groups (intervention or control group). Demographic characteristics will be recorded. All outcome measures will be performed at baseline and postintervention (10 weeks). Clinical assessment will be administered by an independent assessor.

Structural and neurochemical brain biomarkers will be conducted by an MR physicist. Interventions will be provided in 20 physiotherapy sessions over a 10-week intervention period (two visits per week). All participants will be allowed to take pain-relief medications, if necessary, but they will be asked to record the medication type and dose in a medication diary. Participants in the intervention group will be requested to refrain from seeking any other forms of treatment during the trial. All participants will be instructed to continue with their normal daily activities.

Randomisation
All eligible participants will be randomly allocated to either the intervention group, who will receive manual therapy combined with therapeutic exercise, or the control group, who will receive routine physical therapy. The random sequence will be computer-generated using random permuted block sizes of 4 and 6, stratified by age (≤45 or >45)27 and gender (male or female),28 with a 1:1 allocation ratio. The randomisation will be undertaken by an independent person not involved in participant recruitment. Allocation will be concealed in sequentially numbered, sealed, opaque envelopes.

Blinding
The baseline and postintervention assessments will be performed by an independent assessor and an MR physicist who are blinded to participants’ group allocation. Physiotherapists who conducted the intervention will be blinded to outcome assessments throughout the trial. The physiotherapists and participants cannot be blinded to the interventions.

Physiotherapist training and treatment fidelity
Intervention programmes will be provided by physiotherapists who have at least 7 years of clinical experience in cervical musculoskeletal physiotherapy and are experienced in the trial interventions. The physiotherapists will receive 3 days of training (ie, the elements of treatment and home exercise programmes, clinical decision-making and study protocols) to enhance standardisation of the treatment programmes. Training will be provided by a qualified and experienced musculoskeletal physiotherapist. Trial physiotherapists will also be provided with written treatment manuals. Participant case notes will be used to monitor the trial physiotherapists’ fidelity to the intervention procedures.

Intervention group
The intervention group will receive manual therapy (cervical mobilisation) and therapeutic exercise programme (two visits per week) for 10 weeks and each session will last approximately 30–40 min.29 30 Treatment will begin within 1 week following the baseline assessment. Cervical mobilisation refers to the use of low-velocity passive mobilisation techniques as described by Maitland et al.31 Cervical mobilisation (ie, passive accessory and physiological movement techniques) will be applied to
the symptomatic cervical segments, based on the physiotherapist’s clinical examination. Therapeutic exercise programmes are considered according to previous studies.29 32 33 This includes specific exercises for cranio-cervical and cervical flexors and extensors (ie, movement pattern correction, holding capacity, strength and endurance), axiосcapsular muscles including three parts of the trapezius and serratus anterior muscles (ie, scapular control, strength and endurance) and postural (spinal and scapular) correction. The exercise programme includes non-functional to functional exercises and will be progressed gradually through repetitions, direction and load (table 1). Participants will be asked to perform an exercise programme at home once daily for 10 weeks and record their exercise completions and adverse events (eg, muscle soreness or pain) in an exercise diary. The physiotherapist will check the participants’ exercise diaries and set goals/targets to help maintain adherence and motivation. The elements of treatment and a home exercise programme will be individualised and considered based on the initial and progressive assessment of participant’s pain and exercise performance. Some participants may not reach the final level of each exercise depending on their rate of progression.

Control group
The control group will receive routine physical therapy (eg, modalities, range of motion and/or gentle stretching exercise). Participants will also be asked to record treatments and medications received in a logbook during the trial.

Outcome measures
Primary outcomes
Cortical gray matter
MRI brain images will be obtained using a Philip Ingenia 1.5 Tesla MR machine (Philips Healthcare, Amsterdam, Netherlands) equipped with a SENSE head coil. High-resolution whole-brain T1-weighted will be acquired using a 3D-T1 Turbo Field Echo pulse sequence with the following setting: repetition time (TR)=7.4 ms, echo time (TE)=3.4 ms, field of view (FOV)=256×256mm², voxel size=1.0×1.0×1.0mm³, flip angle=7°, slice thickness=1mm and acquisition time (TA)=6.10 min. A FreeSurf image analysis suite (V.7.2.0) will be used to measure grey matter volume and cortical thickness of whole-brain and regions of interest (ROIs) related to pain processing and integration according to previous studies.2 3 34 35 These include M1, insula, PFC, cingulate cortex, precuneus, periaqueductal grey matter, S1 and thalamus. The ‘recon-all’ pipeline in FreeSurfer will be used to calculate the ROI cortical volumes and thickness with standard settings at baseline and postintervention for all participants. The pipeline produces automated parcellation of the brain structures according to the Desikan-Killiany atlas and the Destrieux atlas. Both atlases are anatomically valid and reliable.36 37

Secondary outcomes
White matter integrity
Diffusion tensor imaging (DTI), a non-invasive imaging technique implemented by MRI will be used to quantify the diffusion properties (directionality and magnitude of water molecules) of whole-brain white matter.38 DTI will be acquired in 32 diffusion-weighted volumes with gradient encoding applied in 32 non-collinear directions (b=1000s/mm²) including one non-diffusion-weighted reference image (n0dir, b=0s/mm²). The two-dimensional diffusion-weighted spin echo (SE)/echo planar imaging pulse sequence with the following parameters will be applied: TR=4065 ms, TE=86 ms, FOV 225×225 mm², voxel size 2.5×2.5×2.5 mm³, flip angle=90°, slice thickness=2.5 mm, TA=9.04 min. All raw images will be visually inspected for artefacts, excessive motion and anatomical abnormalities. DTI data will be analysed by the Functional MRI of the Brain (FMRIB)’s Diffusion Toolbox DTI pipeline of the Oxford Centre for FMRIB Software Library (FSL). The processing pipeline includes the following steps: TOPUP to estimate and correct for susceptibility induced field, brain extraction tool for brain extraction, EDDY for distortion and motion correction, DTIFIT for diffusion tensor fitting, TBSS for aligning FA, a scalar measure of relative diffusion anisotropy and MD, a measure of mean water mobility by using a standard registration algorithm.

Neurochemical biomarkers
The neurochemistry biomarkers will be obtained in different brain regions including dorsolateral PFC (DLPFC), S1, insula and thalamus, using single-voxel proton MR spectroscopy (¹H-MRS) with a 1.5 Tesla, Ingenia, Philip MR machine (Philips Healthcare, Amsterdam, Netherlands) with a SENSE head coil (TR=1000 ms, TE=80 ms, FOV=225×225 mm², voxel size=1.0×1.0×1.0 mm³, slide thickness=1 mm, flip angle=8°, TA=29s). These brain areas are associated with pain and based on previous studies of MRS of brain metabolite levels across various pain pathologies.18 19 39 T1-weighted images in axial, sagittal and coronal planes will be obtained for localising the ¹H-MRS voxels. The point resolved spectroscopy (PRESS) pulse sequence (TR=2000 ms, TE=33 ms) will be used for the examination. The voxel 21×17×18 mm will be used to define the DLPFC, the voxel 10×15×14 mm to define the S1, the voxel 10×22×14 mm to define the insular cortex and the voxel 10×20×13 mm to define the thalamus.18 The brain metabolites measured include NAA; creatine, Cr; Glu/ Gln; myoinositol and Cho).

Neck pain intensity
A VAS will be used to measure participants’ neck pain intensity over the past week. It is a horizontal line with two endpoints that ranges from 0 (no pain) to 100 (worst imaginable pain) mm.40 The VAS has been shown to have high reliability and validity to measure pain.41 42
<table>
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<th>Exercises</th>
<th>Level</th>
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| **Cervical flexors** | 1     | Re-education movement pattern  
SP: supine crook lying  
Gentle and controlled nodding (craniocervical flexion, CCF) action, facilitated with eye movement (target level 22–30 mm Hg). | 10 reps                       |
|                  |       | **Holding capacity**                                                       |                               |
|                  |       | SP: supine crook lying  
CCF action with holding steadily (22–30 mm Hg)                              | 10 s holds × 10 reps          |
|                  | 2     | Coordinated activation of deep/superficial cervical flexors  
SP: upright sitting  
Controlled head movement throughout extension range and return to a neutral position | 10 reps                       |
|                  | 3     | Strength and endurance of cervical flexors  
SP: upright sitting  
Isometric CCF in a progressive range of cervical extension  
Lifting the head off the wall while keeping CCF action (the chair up to 30 cm away from the wall) | 10 s holds × 10 reps          |
|                  |       | SP: supine crook lying  
Lifting the head off a pillow while keeping CCF action (2, 1 and 0 pillows as per participant’s capacity) | 10 s holds × 10 reps          |
|                  | 4     | Progressive training  
SP: upright sitting  
Isometric CCF action against a resistance band (loads depending on participant’s capacity) | 10 s holds × 10 reps          |
|                  |       | SP: supine crook lying  
Lifting the head off a bed against the resistance band while keeping CCF action (loads depending on participant’s capacity) | 10 s holds × 10 reps          |
| **Cervical extensors** | 1     | Re-education movement pattern  
SP: prone on elbows/four-point kneeling  
Craniocervical extension while maintaining the cervical spine in a neutral position  
Craniocervical rotation (<40°) while maintaining the cervical spine in a neutral position  
Cervical extension while keeping the craniocervical region in a neutral position | 5 reps × 3 sets                |
|                  | 2     | Cocontraction of deep cervical extensors/flexors  
SP: upright sitting  
Isometric cervical rotation (left and right sides), facilitated with eye movement | 5 s holds × 5 reps            |
|                  | 3     | Strength and endurance of cervical extensors  
SP: prone on elbows/four-point kneeling  
Isometric hold in a progressive range of cervical extension | 10 s holds × 10 reps          |
|                  | 4     | Progressive training  
SP: prone on elbows/four-point kneeling  
Isometric hold in the range of cervical extension against light weights attached to head | 10 s holds × 10 reps          |
|                  |       | SP: upright sitting  
Cervical extension against a resistance band (loads depending on participant’s capacity) | 10 s holds × 10 reps          |
| **Axio-scapular muscles** | 1     | Re-education of scapular movement control  
SP: side-lying with arm elevate 140°/upright sitting  
Passive scapular repositioning  
Active scapular repositioning | 10 reps                       |
|                  |       | **Holding capacity**                                                       |                               |
|                  |       | SP: side-lying with arm elevated 140°/upright sitting  
Isometric hold of active scapular repositioning | 10 s holds × 10 reps          |

Continued
Neck pain and disability
The Neck Disability Index (NDI) will be used to measure disability due to neck pain.43 It consists of 10 questions including pain intensity, headache, concentration, reading, sleeping, driving, work, personal care, lifting and recreation. Each item has a score ranging from 0 to 5, which 0 indicates the highest level of function and 5 the lowest level of function. A total score is obtained with the sum of the responses and then expressed as a percentage. The NDI has been shown to have high validity and reliability.43 44

Anxiety and depression
The Hospital Anxiety and Depression Scale (HADS) will be used to assess symptoms of anxiety and depression (HADS-A and HADS-D) over the past week.45 It consists of two subscales, HADS-A and HADS-D subscales, each with seven items. Each item is scored from 0 to 3. A total score for each subscale ranges from 0 to 21, with higher scores indicating worse symptoms. The HADS has been shown to be a reliable and valid tool for screening anxiety and depression in a wide variety of clinical groups.46–48

Cervical range of motion
A CROM goniometer (Performance Attainment Associates, USA) will be used to assess CROM in flexion, extension, left-right lateral flexion and left-right rotation. Each direction will be performed three times and the average value will be used for analysis. Any pain occurred will also be recorded on a 0–10 Numerical Rating Scale. The
CROM has been shown to be a clinically reliable and valid tool for measuring CROM.49

Cervical muscle strength
Cervical muscle strength will be measured in sitting using a handheld dynamometer (DynaMo, VALD Performance, QLD, Australia). The dynamometer resistance pad will be placed under participants’ mandible with the head and neck positioned in a neutral position. Participants will be instructed to nod their head (craniocervical flexion direction) against the resistance pad as hard as possible for 3–5s, three times. Two practices will be given for familiarisation prior to testing. Standardised verbal encouragement will be given to all participants during the test. The highest value will be used for analysis.

Sample size
There is no evidence for the effects of manual therapy combined with therapeutic exercise on brain structures in patients with chronic non-specific neck pain. Sample size calculation was, therefore, based on a previous study examining pretreatment to post-treatment changes in volumes for brain structures following interdisciplinary pain management programme (including physical therapy) in patients with chronic pain.13 The study showed a significant increase in total grey matter brain volume (mean percent change=0.39, p<0.001) after the programme. In addition, the study demonstrated that the average percent increase in total brain volume was greater for nonopioid users (mean percent change=0.51) compared with opioid users (mean percent change=0.21), which trended towards statistical significance (p=0.079). Assuming the SD of 0.4 for changes in brain volume,13 and using an unpaired t-test with 80% power and a 5% confidence level, the optimum number of 21 participants per group is required. To allow for 20% drop-out rate, a total sample size of 52 participants (26 per group) will be recruited for this trial.

Statistical analysis
Descriptive statistics will be used to describe demographic data and characteristics of participants at baseline and postintervention. Differences between the intervention and control groups in grey matter (volume and cortical thickness) of both whole-brain and ROIs will be applied. Voxel-wise statistics of the DTI parameters (FA and MD) for group differences will be tested in a GLM framework with the unpaired t-test, using the FSL randomise tool with a non-parametric permutation testing. Correction for multiple comparisons across voxels will be performed using the threshold-free cluster enhancement method and statistical maps will be obtained with family-wise error (FWE) corrected p values less than 0.05.

Analysis of the secondary outcomes will be performed using an intention-to-treat approach. Multiple imputation will be performed to handle missing data. Differences between the intervention and control groups will be analysed with an analysis of covariance model using the baseline value as one of the covariates. Effect size will be calculated by using partial eta squared ($\eta^2_p$): small≥0.01, moderate≥0.06 and large≥0.14.50 A significance level will be set at 0.05. All statistical analyses will be conducted by using the SPSS package, version 22 (IBM Corporation, Armonk, NY).

ETHICS AND DISSEMINATION
The study protocol was approved by the institution’s ethical review committee for research in humans (No. AMSEC-63EX-101).

The procedures will be conducted according to the Declaration of Helsinki. Written informed consent will be obtained from all participants before enrolment in the study. Participants’ privacy will be respected, and identification of the included participants will be made by a unique code number. We will submit a manuscript describing the results in a peer-reviewed publication and present in the conference presentation.

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Contributors Conceptualisation and design: RC, MS, SK, SS, KW and SU. Methodology: RC, MS, SK, SS, KW and SU. Drafting and revising the manuscript: RC, MS and SU. All authors read and approved the final manuscript.

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