CHAPTER 3

MATERIAL AND METHODS

3.1 Research design

The within subject repeated measure design was used in this study. Participants were selectively randomized for both of a wrist, which received interventions, and the order of interventions: placebo ultrasound, pulse ultrasound and continuous ultrasound. A crossover study design was used to account of the order of interventions for inter-subject variability. For consistency throughout the study, each participant received one ultrasound condition a day at the same time for four consecutive days. All of subjects, physiotherapist providing ultrasound treatment and the researcher collecting thermal detection thresholds data were unaware of which intervention was being applied, thus making it a double-blind study. The experimental procedures were approved by the Faculty of Associated Medical Sciences Ethics Committee, Chiang Mai University, prior to data collection.

3.2 Participants

Thirty healthy female volunteers ranging in age from 18 to 30 years were recruited from students and support staff of Chiang Mai University who responded from a general notice and word of mouth. General information about the intent of the study was provided to all participants, but the expected results did not discuss before completion of their testing. All patients signed a consent form before entry to the study and were requested to avoid from taking caffeine/alcohol and undertaking

physical activity within 1 hour before data collection. Demographic information and previous pain experience were collected for each participant.

Participants were excluded from the study if they had any known or suspected upper extremity and/or cervical pathology, diabetes mellitus, diseases affecting sensory nerves, musculoskeletal disorders, previous surgery of upper limbs and/or cervical, present pain, taking any medication, smoking and having ultrasound contraindications such as vascular abnormalities, deep vein thrombosis, emboli, artherosclerosis, haemarthrosis, haematomas, uncontrollable haemophillia, malignant tumor, cardiac diseases and pregnant.

3.3 Variables

3.3.1 Independent variables

Modes of therapeutic ultrasound

- placebo ultrasound
- pulse ultrasound
- continuous ultrasound

3.3.2 Dependent variables

- Cold detection thresholds (CDTs) (°C)
- Warm detection thresholds (WDTs) (°C)

3.4 Equipments

- Ultrasound unit
- Thermal Sensory Analyzer (Model TSA 2001, Medoc Ltd., Ramat Yishai, Israel)
- Blind fold
- 10 cm^2 Circular template
- Ultrasound gel

3.5 Experimental setup

3.5.1 Ultrasound unit

A regular physiotherapeutic ultrasound unit was used for experimental condition. The transducer with an effective radiation area of 5 cm^2 was connected to the unit. Ultrasound unit was checked and calibrated for frequency, timer, beam symmetry and acoustic output power.

The technical ultrasound parameters were set as followed: Continuous mode with intensity of 1 W/cm^2 during the entire experiment and a frequency of 1 MHz. Pulsed mode with the same intensity (1 W/cm^2) and frequency (1 MHz) of continuous mode with a 1:5 pulsed ratio (20%). Placebo with the technique of ultrasound application, the machine was set for the intensity at 0 W/cm^2. Therefore, no ultrasonic waves emitted into the body. Five minutes were used for each experimental condition.

The ultrasound procedures were in the following orders. Firstly, participants lay supine on a plinth, with the experimental arm exposed from the elbow downward.

The elbow was in extended position and supported on a pillow. Then area of treatment was drawn on volar site of experimental wrist using a 10 cm^2 circular template to enhance standardization, which was twice the size of the radiating area of the ultrasound applicator. An ultrasound transmission gel was thinly spread on the experimental surface. The ultrasound application was screened behind the physiotherapist so that both of participant and physical therapy did not know the mode and intensity of the ultrasound given (Figure 3-1).

Figure 3-1 Position of participant, physiotherapist and research assistant during ultrasound application

The ultrasound was given by a trained research assistant (UP) that was not blinded to the experimental/placebo allocation. However she did not indicate to the participants which treatment she was applying. She told them that the arm was being treated with a different intensity, that there was a possibility of feeling some warmth, and that they were to indicate if they felt any pain during the treatment. The participants could stop the treatment if they want. Ultrasound was applied using

circular technique on the area of treatment by physiotherapist (KB) for 5 minutes. The ultrasound transducer head was continuously moved in a 90° angle to the skin surface to ensure that the majority of the ultrasound beam did not travel parallel through the dermal layer and at a rate of approximately 3 cm/sec for 5 minute in each experimental condition.

3.5.2 Thermal detection thresholds

Thermal detection thresholds in this study are cold detection threshold (CDTs) and warm detection threshold (WDTs) which tested using a computer controlled Thermal Sensory Analyzer (Model TSA 2001, Medoc Ltd., Ramat Yishai, Israel) utilizing a peltier-element-based stimulator. It produces a thermal stimulus and collects response of thermal detection threshold, with an ascending method of limits protocol.

The technical TSA II parameters were set as followed[98, 99]: A 30x30 mm^2 contact thermode was secured on the thenar eminence of the experimental hand to produce warming and cooling temperature. The changing of thermode temperature was set at a rate of 1 ºC/sec from a baseline of 32 ºC and a returning rate of temperature was 10 ºC/sec**.** To avoid any tissue damage, the cut-off temperature for all trials was set at 50 ºC for heat and 0 ºC for cold stimulus. Inter-stimulus intervals of 10 seconds for sensory detection threshold trials were maintained between successive stimuli to avoid either sensitization or habituation of the cutaneous receptors. The testing was carried out in a silent room at 26 °C.

For the thermal perception thresholds testing procedures, participants sat on edge of plinth, with the experimental wrist exposed from the elbow downward. The elbows was flexed to 90° and supported on a pillow. The experimental wrist was in supine position and contacted with thermode, which delivered thermal stimuli, at thenar eminence. The non-experimental hand was in pronate position to click a computer mouse to represent a response of thermal detection thresholds. The participants were not allowed to watch the computer screen. Thus the participants were blindfolded during thermal testing procedure. (Figure 3-2)

Figure 3-2 Thermal detection threshold testing procedure, showing the position of participant and researcher, the placement of the thermode on the thenar eminence and the holding of responding mouse.

To measure cold detection thresholds, the temperature was continuously decreased with a linear rate of 1°C/sec from baseline temperature of 32°C until the

participant indicated, by clicking a computer mouse, the first perception of cold sensation. The thermode then return to 32°C with a linear rate of 10°C/sec and 10 seconds time interval were set between successive trials. To measure warm detection thresholds, the temperature was gradually increased from baseline temperature of 32°C until the participant indicated the first warm sensation perception in the same rate and time interval with CDTs. Finally, the thresholds of the cold detection threshold or warm detection threshold were recorded and defined as the average of the three measurements made for each sensation.

3.6 Experimental protocol

After participants were screened for the suitable criteria and signed a consent form, participants were randomly assigned by blind cards allocation, to receive one of 6 possible orders of presentation of the 3 modes of ultrasound and side of forearm receiving experimental condition (left or right). The familiarization period was provided to each participant one day prior to data collection. Thermal detection testing was demonstrated over volar forearm ipsilateral to the experimental wrist to make a better understanding of procedure.

Two series of experiments were performed. The first series was assigned to assess stability of thermal perception thresholds testing between the three time intervals (immediately 0 minute, 5^{th} minutes, and 24^{th} hours). The second series assessed the effects of therapeutic ultrasound on thermal perception thresholds.

Series 1: Stability of thermal perception thresholds testing on the three time intervals.

Thirty healthy female volunteers participated in this set of experiment. Each subject received thermal perception thresholds testing on the experimental wrist without ultrasound application. Cold detection thresholds and warm detection thresholds were recorded at 0 minute, 5^{th} minutes, and 24^{th} hours, respectively. These three time intervals represented pre-ultrasound, post-ultrasound immediately, and time to next ultrasound condition, respectively.

Series 2: The effects of therapeutic ultrasound on thermal perception thresholds.

Thirty healthy female volunteers, the participants from series 1, completed series 2. From randomization procedure, each participant received side of wrist receiving experimental condition and one of six possible orders of presentation of the three modes of ultrasound (Appendix A). Details of each mode are summarized below.

- Placebo ultrasound: participants received the technique of ultrasound application in a similar manner to the treatment procedures (frequency=1 MHz, duration=5 minutes), except that the intensity was zero (0 W/cm^2).
- 20%Pulsed ultrasound: participants received an active ultrasound that were applied with a frequency of 1 MHz, 20% of intensity of 1 W/cm^2 (Spatial Average Temporal Peak (SATP) intensity = 1 W/cm^2, Spatial Average Temporal Average (SATA) intensity = 0.2 W/cm^2).

- Continuous ultrasound: participants received an active ultrasound that were applied with a frequency of 1 MHz, an intensity of 1 W/cm^2 (SATP= 1 W/cm^2, SATA= 1 W/cm^2).

The participants received each of ultrasound condition followed the ultrasound procedures. Five minutes treatment period were used for each ultrasound condition. Each participant underwent all 3 ultrasound conditions within 3 consecutive days, receiving once a day, always at the same time. The thermal perception thresholds testing procedures, same as series 1, were used to test before and again immediately after completion of each intervention. Then cold and warm detection thresholds were recorded.

3.7 Statistical analysis

Demographic data of participants were analyzed using descriptive statistics. All thermal threshold values were expressed as change from the baseline temperature of 32°C. Both of cold and warm detection thresholds were calculated as absolute of 32°C minus recorded value [72, 99]. These absolute values of the threshold were normally distributed in log-transformed data with the Shapiro-Wilk test (P>0.05) and were transformed logarithmically before statistical analysis. Log- transformed data were used to analyze data from series 1 and series 2[25, 26, 98, 99].

Series 1 Differences between the three time intervals of cold detection thresholds and warm detection thresholds were analyzed by a separate repeated-measures analysis of variance (ANOVA) for each thermal threshold.

Series 2 Differences in the baseline values, which were assessed before each intervention, were analyzed by a separate repeated-measures analysis of variance (ANOVA) for each thermal threshold. Differences in the changes of threshold that occurred from pre to post-intervention were analyzed with two tailed paired samples t tests for each thermal threshold. Log- transformed data of the thresholds were used to calculate change in threshold of control, placebo ultrasound, pulse ultrasound, continuous ultrasound as threshold post-intervention minus baseline. Thus, positive log-thresholds values for change always represented an increase in the threshold and therefore a decrease in sensitivity, and negative log-thresholds values always represented a decrease in threshold and therefore an increase in sensitivity. Differences in the changes in log- transformed data of the thresholds that occurred (post- intervention minus baseline) were therefore investigated with a separate repeated-measures analysis of variance (ANOVA) for CDTs and WDTs. The Statistical Package for the Social Sciences (SPSS 10.0 for windows) was used to analyze all data in this study. In all tests, a significance level of $P < 0.05$ was used to reject the null hypothesis of no difference between conditions.