Effect of acupressure on postpartum low back pain, salivary cortisol, physical limitations, and depression: a randomized controlled pilot study

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Abstract

OBJECTIVE: To investigate the effect of acupressure on postpartum low back pain (LBP), salivary cortisol, physical limitations, and postpartum depression.

METHODS: Participants were 70 postpartum women who were randomly assigned to either an intervention (n = 35) or a control (n = 35) group. The intervention group received 10 acupressure sessions (1 session per day, 5 days per week). The control group received 10 sham acupressure sessions. Outcomes were assessed using a visual analogue scale (LBP intensity), salivary cortisol values (LBP biomarker), and Chinese versions of the Roland–Morris Disability Questionnaire (daily activity limitations), Oswestry Disability Index (physical activity limitations), and the Edinburgh Postnatal Depression Scale (postpartum depression).

RESULTS: Participants in the intervention group had significantly lower levels of LBP intensity, daily activity limitations, physical activity limitations, and postpartum depression than those in the control group. There was no significant between-group difference in salivary cortisol.

CONCLUSIONS: Acupressure may reduce postpartum LBP intensity and limitations in daily and physical activity, and alleviate postpartum depressive symptoms. Acupressure should be offered in postpartum care settings as an alternative treatment for postpartum women with LBP.

Keywords: Acupressure; Low back pain; Postpartum

INTRODUCTION

Low back pain (LBP) is a common complaint in postpartum women. In a cohort study , about 48% of Australian women report LBP in the first 3 months postpartum.[1] In one study, 44% of women had experienced LBP on the fourth day postpartum and 31.5% continued to have LBP at 3 months postpartum.[2] In Taiwan, 31.5% to 35.7% of primiparas reported LBP within 3 years of delivery.[3] Hormonal and physical changes during pregnancy make pregnant women vulnerable to LBP. Relaxin, a hormone present during pregnancy that is responsible for muscle softening and increased mobility of the joints, may contribute to LBP.[4] From the first to the third trimester, increased weight gain exaggerates lumbar lordosis, causing excess pressure in the spine and LBP.[5,6] Although these hormonal and physical changes gradually subside after birth, some women continue to experience postpartum LBP. There are several additional determinants of postpartum LBP, such as the presence of back pain during pregnancy, heavy physical work, multiple pregnancy,[7] obesity,[8] smoking,[2] and epidural or spinal anesthesia for labor or caesarean section.[3] Older (≥35 years) and primipara status are also associated with increased LBP up to 1 month postpartum.[5] After delivery, infant care activities such as lifting and carrying may also contribute to LBP.[9]

The hypothalamic–pituitary–adrenal axis (HPAA), which generates cortisol, is stimulated by physical and psychological stress.[10] Pain creates a state of stress; this affects HPAA activity and increases cortisol secretion.[11] Cortisol may be an objective biomarker for pain; however, studies on the cortisol response of patients with chronic LBP are inconsistent. A few studies have shown lower[12] or unaltered[13] cortisol levels in patients with chronic LBP compared with healthy subjects. In one study, there was a non-significant increase in salivary cortisol level in response to painful heat stimuli between patients with chronic LBP and a healthy group.[12]

Musculoskeletal pain can lead to negative psychological states, such as depression.[14, 15] Pelvic girdle pain is considered a subgroup of LBP. One report showed that depressive symptoms at 3 months postpartum were more prevalent among women with pelvic girdle pain and lumbar pain than among those without pain.[16] Another study indicated that back pain may be a risk factor for postpartum depression.[17] Conversely, poor psychological health may also be associated with poor prognosis for musculoskeletal pain. Women who experienced emotional distress during

pregnancy had an increased risk of persistent pelvic girdle pain 6 months after delivery.[18] In postpartum women, a combination of LBP and depression may be overwhelming and may compromise their ability to care for their babies,[8, 19] limit physical functions and daily activities, and reduce self-efficacy and quality of life.[15]

Interventions to relieve or reduce LBP in postpartum women are therefore important. Although pharmacological interventions for LBP are available, owing to breastfeeding and infant care concerns, treatments for postpartum women are generally conservative and focus on exercise-based interventions and alternative strategies. For instance, one clinical trial found that women with pelvic girdle pain who received 20 weeks of specific stabilizing exercise had decreased pain intensity and increased quality of life at 1 year postpartum.[20] Another study showed that the most popular interventions for women with LBP at 12 months postpartum were physical therapy, chiropractic and naprapathic treatment, and other treatments such as acupuncture.[21] Chinese women particularly prefer to use complementary and alternative medicine (which includes acupressure, acupuncture, massage therapy, and reflexology) in the postpartum period for pain relief and relaxation.[22]

Acupressure improves physical functions by applying controlled irritation to active meridian points. The mechanisms underlying the effect of acupressure are not entirely understood. Acupressure may stimulate the somatic, autonomic nervous, and neuroendocrine systems, and increase the production of endogenous opioids; this activates the body's self-healing abilities and reduces pain.[23,24] Several researchers have reported that acupressure can relieve back pain. Participants in one Taiwanese study had significantly reduced LBP 6 months after receiving six 15-minute acupressure sessions over a 4-week period.[25] An Iranian study showed that 50 female nurses who received acupressure for 4 weeks had significantly lower pain scores than a sham acupressure group.[26] Another study reported that acupressure on the acupoint Shènshū (BL 23) and cupping therapy given to primiparous women with postpartum LBP reduced their pain intensity effectively compared with a control group.[27]

Although the effect of acupressure in reducing LBP has been explored, the relationship between acupressure and LBP in postpartum women has not been examined. Further, no Taiwanese studies have used objective biomarkers to assess pain response to acupressure in postpartum

women with LBP. Therefore, the aim of this study was to investigate the effectiveness of an acupressure program in women within 1 month postpartum. The primary outcomes were as follows: subjective assessment of LBP using a visual analogue scale (VAS), and assessment of cortisol as an objective biomarker for pain. The secondary outcomes were daily activity and physical activity limitations, and depression.

METHODS

Definition of LBP

LBP was defined as self-reported pain in the lumbar area between the 12th rib and the gluteal fold, characterized as a dull pain, more pronounced in forward flexion, and with associated restriction in spine movement.[28-30]

Study design

This was a randomized, double-blind, controlled study. It was conducted at a postpartum care center within a medical center in southern Taiwan between November 2017 and July 2018. The study was approved by the Institutional Review Board of Chang Gung Medical Foundation (IRB permit no. 201700427B0C601). Randomization was based on a 1:1 allocation ratio. Sample size was estimated using GPower software (GPower3.1.9.2, Heinrich-Heine-Universitat Dusseldorf, Germany). The estimated sample size ($\alpha = 0.05$, power = 0.95, and effect size = 0.8) was 35 patients per group.

Study participants

In Taiwan, an increasing number of postpartum women are admitted to postpartum care centers after hospital discharge. In these care centers, postpartum women and their babies are cared for by nursing staff for about 1 month; in Taiwan, this period is called "Tso Yueh-tzu" or "confinement." A convenience sampling method was used to recruit participants admitted to the postpartum care center. A research assistant used a VAS and a picture of LBP location to screen participants according to the study criteria. Those whose VAS score for LBP was more than or equal to 1 were recruited. Eligible participants had (1) delivered a viable baby within 1 month, (2) were ≥20 years old, (3) had an LBP score ≥1 on the VAS, and (4) planned to stay in the center for at least 2 weeks. Exclusion criteria were (1) systemic diseases, cancer, psychiatric diseases, and (2) receiving treatment for severe pain by a physician. Informed consent was obtained from all participants before randomization. Finally, 70 participants were enrolled and randomly allocated to either an acupressure group (n = 35) or a control group (n = 35). All participants

and the researcher who collected and analyzed the data were blinded.

Intervention

The intervention group received 10 acupressure sessions: 1 session per day for 5 days per week. We selected this intervention frequency for two reasons. First, postpartum women generally remain in the postpartum care center for approximately 1 month; this permits 10 daily acupressure sessions during their stay in the center. Second, previous studies indicate that acupoint stimulation tends to show an effect after an average of 9.6 acupressure sessions.[31] The following 10 bilateral acupoints were selected according to traditional Chinese medicine principles, the WHO standardized acupuncture point location guideline,[32] and literature reviews: Shènshū (BL 23), Dàchángshū (BL 25), Guānyuánshū (BL 26), Wěiyáng (BL 40), and Sānyīnjiāo (SP 6). Each acupressure session was performed by the study nursing staff. Treated started at Shènshū (BL 23), followed by the other body points, then the leg points. Participants received acupressure in a prone position. Acupressure was administered for 2 minutes on five acupoints per day, bilaterally (The total session lasted for 10 minutes), and 5 times a week.

Control group/sham acupressure group

The control group received 10 sham acupressure sessions. These featured the same acupoints as the intervention group, but stimulation was given 2 inches above each acupoint. Acupressure was administered for 10 minutes per day on five acupoints, bilaterally, and 5 times a week.

Measurement

The VAS was used as a subjective measure of LBP and cortisol was measured as an objective biomarker for LBP. Limitations of daily and physical activity, and postpartum depression, were measured at baseline and following the 2-week intervention (the day after the 10th session).

Primary outcome

LBP intensity

Subjective pain intensity was scored on a 0–10 cm VAS. The distance was measured in cm from zero to the point the subjects had marked on the VAS.[33]

A biomarker of LBP

Salivary cortisol values were used as an objective biomarker for pain. Golden has reported that salivary cortisol peaks in the morning and

reaches its nadir in late evening.[34] Salivary samples were obtained from each participant in the morning and evening at baseline and after the intervention (the day after the 10th session). In the morning, participants were asked to rest in bed on an empty stomach (although they could drink boiled water) and to not brush their teeth so that saliva could be collected. In the evening, saliva was collected before dinner (about 18:00–19:00). Each time, we collected 1 cm³ of saliva from each participant in a 10 cc sterile container. Salivary cortisol was measured using a Salimetrics® Cortisol Enzyme Immunoassay Kit (Item No. 1-3002, Salimetrics LLC, State College, PA, USA) according to the manufacturer's instructions and a previous study.[35] This kit is a competitive immunoassay used for quantitative measurement of salivary cortisol. Salivary cortisol values were expressed in nmol/L. All samples were analyzed in duplicate for salivary cortisol.

Secondary outcomes
Limitations in daily activity and physical activity

Postpartum women with LBP have decreased physical activity, and therefore restricted daily activities. In 1998, the International Program on Primary Care on Back Pain proposed a standardized set of outcome measurement questionnaires for LBP studies to facilitate data collection and the interpretation and comparison of aggregate data. This set of questionnaires contains the Roland–Morris Disability Questionnaire (RMDQ) to assess daily activities and the modified version of the Oswestry Disability Index (ODI) to assess physical activities.[36] The Chinese version of the RMDQ was used to score daily activity limitations[37] and the Chinese version of the ODI (version 2.1) was used to score physical activity limitations.[38]

The RMDQ is a 24-item measure of the impact of back-related pain on daily activity/function. Participants are asked to select "yes" or "no" to indicate if a daily activity or function is affected; a score of 1 is assigned to "yes" and 0 to "no." The total score ranges from 0 to 24; higher scores indicate a greater limitation in daily activity. The RMDQ has demonstrated good reliability and validity[37]. The ODI comprises one item on pain and nine items on physical activities of daily living (personal care, lifting, walking, sitting, standing, sleeping, sex life, social life, and traveling). Each item is scored from 0 (able to perform) to 5 (unable to perform). ODI items 8 and 10 were removed as they are not culturally relevant to postpartum women in Taiwan. Postpartum women in Taiwan

are not likely to engage in sex or travel 4–6 weeks after childbirth. Summed scores range from 0 to 40; higher scores indicate greater limitation in physical activity. The ODI has demonstrated good reliability and validity.[38, 39]

Postpartum depression

The Edinburgh Postnatal Depression Scale (EPDS) developed by Cox, Holden, and Sagovsky[40] was used to assess postpartum depression symptoms. Scores range from 0 to 30; higher scores indicate greater postpartum depression. The EPDS has been validated and has demonstrated suitability for postpartum depression screening.[41, 42]

Statistical analyses

All analyses were performed using SPSS 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY, USA). The chi-square test and the independent t-test were conducted to test differences in the baseline characteristics between the two groups. Intention-to-treat analysis was used. We used the paired t-test to investigate within-group differences before and after the intervention. We used the independent t-test and analysis of covariance (ANCOVA) to compare differences in outcome measures between the two groups. The significance level was set at $P<0.05$.

RESULTS

A total of 70 participants were recruited and randomly assigned to the acupressure group (n = 35) or the control group (n = 35). All participants completed the study (Figure 1). The two groups did not differ significantly in baseline demographic characteristics (Table 1) or baseline outcome measures (VAS, salivary cortisol values in the morning and evening, RMDQ, ODI, and EPDS scores) (Table 2).

Primary outcome

1.LBP intensity: VAS

Post-intervention VAS scores in both groups were statistically lower than baseline scores. The mean VAS score post-intervention was lower in the acupressure group than in the control group (1.14 ± 0.91 and 3.11 ± 1.16, respectively); indicating that subjects in the acupressure group had a lower LBP intensity than those in the control group. We used ANCOVA to compare post-intervention covariates after adjusting baseline score covariates of the two groups ($F = 88.67$, $P<0.0001$) (Table 2). The post-intervention adjustment mean VAS scores in the acupressure group and the control group were 1.17 and 3.09, respectively.

2.LBP biomarker: salivary cortisol values

Morning salivary cortisol values

There was no significant change in morning salivary cortisol values in both groups from baseline to the end of the intervention ($P = 0.94$ and 0.43, respectively). Mean morning salivary cortisol values decreased in the acupressure group, but increased in the control group (6.03 ± 4.04 and 7.45 ± 6.36, respectively). The ANCOVA analysis comparing the two groups showed no significant difference after adjusting for baseline score covariates ($F = 1.22$, $P = 0.27$) (Table 2).

Evening salivary cortisol values

There was no significant change in evening salivary cortisol values in both groups from baseline to the end of the intervention ($P = 0.39$ and 0.24, respectively). Mean evening salivary cortisol decreased in the acupressure group and the control group (3.16 ± 2.05 and 3.30 ± 2.22, respectively). The ANCOVA analysis comparing the two groups showed no significant difference after adjusting for baseline score covariates ($F = 0.11$, $P = 0.75$) (Table 2).

Secondary outcomes

1.Limitations in daily activity and physical activity: RMDQ and ODI scores

RMDQ

Post-intervention RMDQ scores for the acupressure group were statistically lower than baseline scores ($P<0.0001$), but there was no significant change in scores from baseline to post-intervention for the control group ($P = 0.92$). The ANCOVA analysis comparing the two groups indicated a significant difference in RMDQ score ($F = 94.11$, $P<0.0001$). This indicated that the acupressure group experienced fewer daily activity limitations than the control group (Table 2). The post-intervention adjustment mean scores in the acupressure group and the control group were 3.23 and 9.82, respectively, indicating that subjects in the intervention group experienced fewer limitations in maternal daily activities.

ODI

ODI scores for both groups were statistically lower post-intervention than baseline scores. The mean post-intervention ODI score was lower in the acupressure group than in the control group (3.03 ± 2.19 and 9.57 ± 4.70, respectively), indicating that the acupressure group experienced fewer physical activity limitations than the control group. The ANCOVA

analysis comparing the two groups showed that the difference remained significant after adjusting for baseline score covariates ($F = 92.74$, $P<0.0001$) (Table 2). The post-intervention adjustment mean scores in the acupressure group and the control group were 3.31 and 9.29, respectively, indicating that acupressure was effective in reducing physical activity limitations.

2.Postpartum depression: EPDS scores

Post-intervention EPDS scores for the acupressure group were statistically lower than baseline scores ($P<0.0001$), but there was no significant change in scores from baseline to post-intervention for the control group ($P = 0.21$). The ANCOVA analysis comparing the two groups showed that the difference remained significant after adjusting for baseline score covariates ($F = 10.86$, $P = 0.002$) (Table 2).

DISCUSSION

This study investigated the effectiveness of acupressure on LBP. There was a significant post-intervention difference in subjective LBP intensity between the intervention and control groups, which indicates that acupressure was effective in decreasing LBP. Compared with the control group, the acupressure group had lower pain intensity after the intervention.

The study findings are in agreement with findings from a previous study, which reported that acupressure on the acupoint Shènshū (BL 23) reduced LBP among postpartum women.[27] This previous study used the short-form McGill Pain Questionnaire to assess pain; there was a significant difference in pain intensity scores between the intervention group and the control group. These findings and our own suggest that acupressure can significantly reduce the subjective pain intensity of postpartum LBP. However, the mean VAS scores of our participants indicated that most had low pain intensity(The scores on the lower end of the 1–10 VAS score range). Further research to confirm the effect of acupressure in relieving LBP in postpartum women with high LBP intensity (i.e., VAS scores ≥5) may be warranted.

We hypothesized that LBP during the postpartum period may alter HPAA activity, which would release cortisol in response to pain. Therefore, cortisol values after acupressure should have been significantly lower in the intervention group than in the control group. However, there were no significant differences in morning or evening

salivary cortisol values from baseline to post-intervention in either group, and no significant between-group difference. Therefore, salivary cortisol values (as an objective pain biomarker) did not indicate that acupressure reduced LBP. A previous study examined cortisol secretion in response to experimentally induced pain stimuli over several time points in three groups of patients: a chronic LBP group, a depression group, and a healthy group. The results showed that induced pain stimuli did not elicit a cortisol response across groups.[12] Some researchers have suggested that the relationship between salivary cortisol and psychological mechanisms in patients with acute LBP (ALBP) versus chronic LBP (CLBP) may differ. Sudhaus et al. defined ALBP as current LBP for less than 6–12 weeks and CLBP as LBP lasting more than 12 months.[43] They found that pain-related coping strategies were negatively correlated with adrenocortical activity in ALBP patients, whereas long-term maladaptive coping strategies contributed to hypocortisolism in CLBP patients. Further investigations should use longitudinal designs to test if cortisol secretion changes in postpartum women are related to ALBP or CLBP. The present findings showed that morning cortisol values were higher than evening values, which supports previous findings that salivary cortisol values reach a high peak about 30 minutes after waking and fall to a minimum in the middle of the night.[34]

Postpartum LBP can partially limit maternal daily activity and physical activity. This study tested the effect of an acupressure program on the degree of limitations in daily and physical activity in postpartum women with LBP. The RMDQ scores of the intervention group were statistically lower post-intervention than those of the control group. Further, ODI scores decreased in the acupressure group post-intervention compared with the control group. Previous research has shown similar positive results from acupressure in improving daily activity limitations owing to LBP,[44] and has demonstrated that physical health problems are associated with postpartum depression in the first 12 months postpartum.[1] Our study showed that acupressure not only reduced subjective LBP intensity, but also alleviated postpartum depression symptoms. Moreover, no adverse events were reported during the intervention period.

This study had several limitations. First, the study was conducted at one postpartum care center. To enhance the representativeness of the sample, future studies should include more study sites (e.g., participants' homes). Second, acupressure is currently not included in routine

postpartum care and treatment. Clinical cost-effectiveness studies should be conducted to determine the viability of offering acupressure treatment. Third, patterns of ALBP and CLBP were not differentiated; additional studies are needed to test the effectiveness of acupressure on ALBP and CLBP that persists from pregnancy. Acupressure is noninvasive, has few adverse side effects, and has minimal requirements in terms of facilities needed. It remains a popular pain-relief intervention for postpartum women.

In conclusion, the present findings indicate that acupressure can reduce postpartum LBP intensity, reduce limitations in daily activity and physical activity, and alleviate postpartum depressive symptoms. Acupressure may be an alternative treatment for postpartum women with LBP.

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Assessed for eligibility (n=81)

Excluded   (n=6)

♦Not meeting inclusion criteria (n=4)

♦Declined to participate (n=2)

♦Other reasons (n= 0)

Randomized (n=70)

Allocation

Allocated to intervention (n=35)

♦ Received allocated intervention (n=35)

♦Did not receive allocated intervention (n=0)

Allocated to intervention (n=35)

♦ Received allocated intervention (n=35)

♦Did not receive allocated intervention   (n=0)

Follow-Up

Lost to follow-up (n=0)

Discontinued intervention0 (n=0)

Lost to follow-up0 (n=0)

Discontinued intervention (n=0)

Analysis

Analysed   (n= 35)

♦Excluded from analysis   (n=0)

Analysed   (n=35)
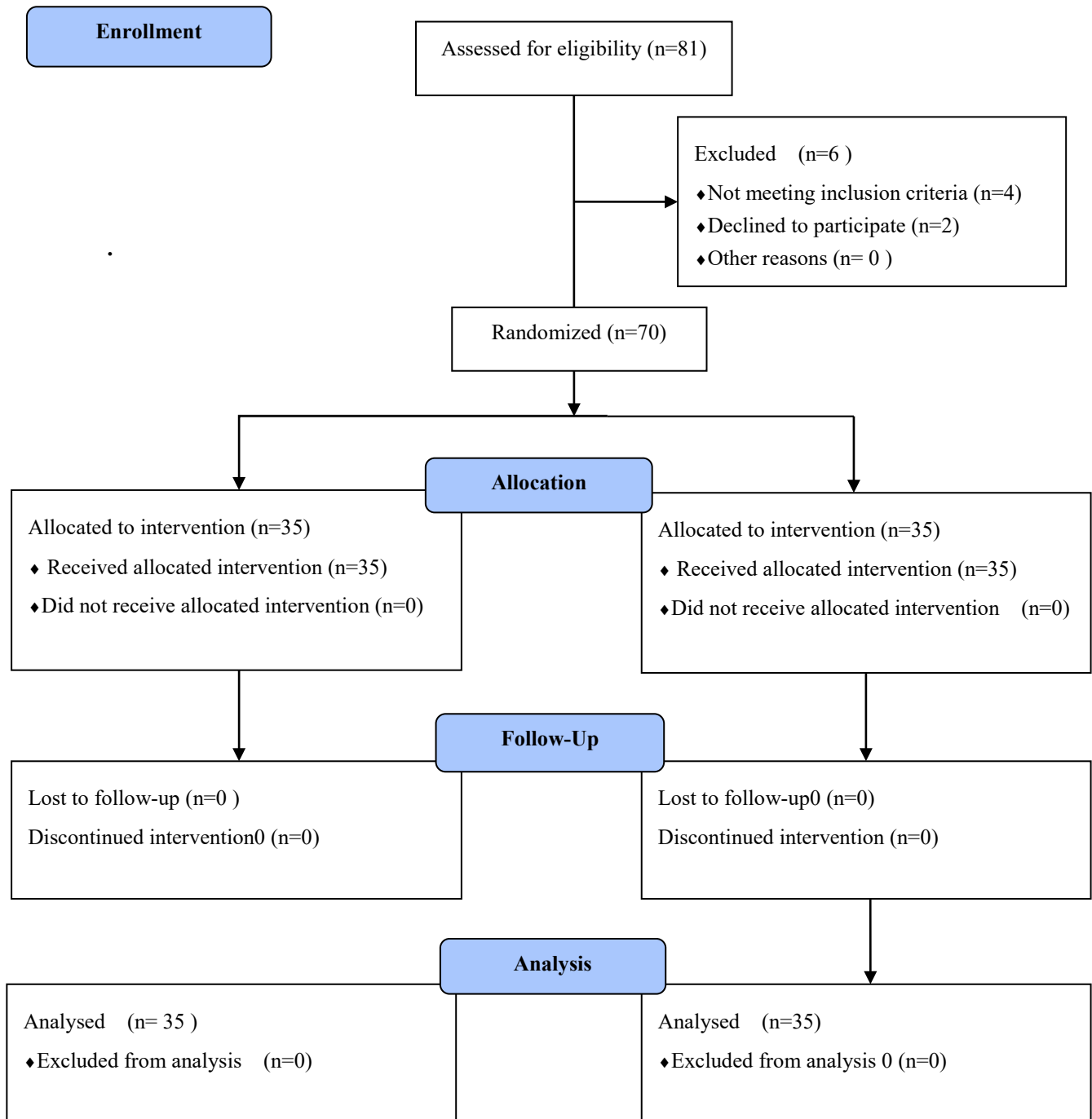
♦Excluded from analysis 0 (n=0)

Figure 1. Participant flow chart

Table 1. Participant demographic characteristics.

Variables	Total (n = 70) M ± SD	[a]Acupressure group (n = 35) M ± SD	[b]Control group (n = 35) M ± SD	t/χ²	p
Maternal age	34.4±4.77	34.8±3.87	34.0±5.57	0.65	0.52
	n (%)	n (%)	n (%)		
Education level				0.26	0.88
High school	9(12.9)	5(14.3)	4(11.4)		
College	42(60.0)	20(57.1)	22(62.9)		
Master and above	19(27.1)	10(28.6)	9(25.7)		
Employment				3.05	0.08
no	26(37.1)	9(25.7)	17(48.6)		
yes	44(62.9)	26(74.3)	18(51.4)		
Delivery				1.56	0.21
Vaginal delivery	45(64.3)	25(71.4)	20(57.1)		
Caesarean	25(35.7)	10(28.6)	15(42.9)		
Obstetrical analgesia				6.08	0.11
No analgesia	36(51.4)	16(45.7)	20(57.1)		
spinal	22(31.4)	10(28.6)	12(34.3)		
General	1(1.4)	0(0)	1(2.9)		
Epidural	11(15.8)	9(25.7)	2(5.7)		
Parity				2.10	0.15
Primipara	40(57.1)	17(48.6)	23(65.7)		
Multipara	30(42.9)	18(51.4)	12(34.3)		
Feeding type					
Breastfeeding only	32(45.7)	15(42.9)	17(48.6)	0.23	0.63
Mixed feeding	38(54.3)	20(57.1)	18(51.4)		
[c]**BMI (kg/m2)**				3.20	0.20
< 23.9(Normal)	38(54.2)	22(62.9)	16(45.7)		
24-26.9(Over weight)	16(22.9)	8(22.9)	8(22.9)		
27 (obesity)	16(22.9.)	5(14.2)	11(31.4)		

Notes: [a]Acupressure group: Received 10 acupressure sessions; [b]Control group: Received 10 sham acupressure sessions. [c]BMI: Body Mass Index(Classification followed the Taiwanese government guidelines.)

Table 2. Comparisons of outcome measures pre- and post-intervention in both groups.

Measure	[a]Acupressure group (n = 35) M ± SD	[b]Control group (n = 35) M ± SD	Independent t	[g]p	F for ANCOVA	[h]p
[c]VAS					88.67	< 0.0001
Pre-intervention	3.83±1.12	3.91±1.17	-0.31	0.76		
Post-intervention	1.14±0.91	3.11±1.16	-7.92	< 0.0001		
paired t	17.65,	4.28				
p	<0.0001	<0.0001				
Salivary cortisol level(morning) (nmol/L)					1.22	0.27
Pre-intervention	6.12±5.61	6.25±5.49	-0.09	0.93		
Post-intervention	6.03±4.04	7.45±6.36	-1.11	0.27		
paired t	0.07	-0.85				
p	0.94	0.43				
Salivary cortisol level(evening) (nmol/L)					0.11	0.75
Pre-intervention	3.63±2.13	3.97±2.47	-0.63	0.53		
Post-intervention	3.16±2.05	3.30±2.22	-0.27	0.79		
paired t	0.86	1.19				
[i]p	0.39	0.24				
[d]RMDQ					94.11	< 0.0001
Pre-intervention	9.31±4.14	10.06±4.04	-0.76	0.45		
Post-intervention	3.06±1.92	10.00±4.43	-8.51	< 0.0001		
paired t	9.59	0.11				
p	<0.0001	0.92				
[e]ODI					92.74	< 0.0001
Pre-intervention	9.80±4.53	10.80±4.84	-0.89	0.38		
Post-intervention	3.03±2.19	9.57±4.70	-7.47	< 0.0001		
paired t	10.80	2.58				
p	<0.0001	0.01				
[f]EPDS					10.86	0.002
Pre-intervention	9.37±4.48	8.97±4.62	0.37	0.71		
Post-intervention	6.34±2.87	8.09±3.84	-2.15	0.04		
paired t	-7.99	-1.28				
p	< 0.0001	0.21				

Notes: [a]Acupressure group: Received 10 acupressure sessions; [b]Control group: Received 10 sham acupressure sessions. [c]VAS: The Visual Analogue Scale; [d]RMDQ: The Roland and Morris Disability Questionnaire; [e]ODI: The Oswestry Disability Index; [f]EPDS: The Edinburgh Postnatal Depression Scale. [g]The independent t-test analysis comparing the two groups; [h]The ANCOVA analysis comparing the two groups; [i]The paired t-test analysis comparing before and after the intervention.