

Comparing the Effect of Transcutaneous Electrical Nerve Stimulation (TENS) At Hugo and Sanjiao Acupoints on Pain Intensity during the First Stage of Labor in Nulliparous Women: A Randomized Clinical Trial

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ARTICLE INFO	ABSTRACT
Article type: Original article	Background & aim: Effective pain management to reduce labor pain is considered as one of the most important issues in making vaginal delivery as a desirable option. The present study was performed to compare the effect of transcutaneous electrical nerve stimulation (TENS) at Hugo and Sanjiao acupoints on labor pain intensity during the first stage of labor in nulliparous women.
Article History: Received: 18-Jan-2024 Accepted: 06-Aug-2024	Methods: This randomized clinical trial was performed on 129 nulliparous women referred to one Educational and Treatment Center in Zanjan, Iran. The participants were randomly assigned into three 41-member groups: the Hugo, the Sanjiao, and the control. Data collection tools included VAS scale and labor management form. The intervention was conducted by applying a TENS device at Hugo (LI4) or Sanjiao (SP6) acupoints since the beginning of active phase (dilatation of 4-5 cm) until dilatation of 10 cm. Pain scores were assessed at three dilation points: before intervention, at dilation 6 and 9 cm using VAS scale. The data analysis was carried out by R software (version 4.2.2) using ANOVA or Kruskal-Wallis tests. In addition, Dunn's test was used to compare variables.
Key words: Labor Pain Hugo Sanjiao Transcutaneous Electrical Nerve Stimulation (TENS)	Results: There was no significant difference among the three groups regarding the pre-intervention pain intensity ($P=0.200$). However, the Hugo and Sanjiao groups experienced significantly less pain in dilatation 6 and 9 cm compared to the control group ($P<0.001$). Moreover, no significant difference was observed between Hugo and Sanjiao groups regarding the pain intensity ($P\geq0.05$).
	Conclusion: The use of TENS device at the acupoints can be effective in decreasing labor pain intensity.

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Introduction

Labor pain is among the most acute and severe pains experienced by women during her life, the intensity of which depends on many physiological, anatomical, social, and cultural factors, as well as women's previous experiences (1-2). Labor pain is known as one of the biological signs of the onset of labor although

uncontrolled pain can be associated with anxiety, traumatic delivery, and fetal and neonatal asphyxia, as well as losing psychological control (3-4). The American Society of Anesthesiologists (ASA) and American College of Obstetricians and Gynecologists (ACOG) consider labor pain as one of the treatment indications (5).

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Labor pain control methods (pharmaceutical and non-pharmacological interventions) seek to help women improve pain tolerance and make labor desirable (6). Pharmacological analgesia causes complications such as maternal hypotension, reduced uterine perfusion, fetal bradycardia, fever, itching, more need for oxytocin, and higher patient costs, as well as prolonging the second stage of labor and increasing the need for cesarean section (7). However, many non-pharmacological pain relief approaches are non-invasive, safe, cheaper, and easier to use that enhances women's satisfaction with childbirth experience (8).

Acupuncture, acupressure, and transcutaneous electrical nerve stimulation (TENS) as non-pharmacological labor pain relief methods can reduce labor pain (9). The mechanism of action of acupressure can be explained based on various theories, some of which suggest that acupressure changes the structure of the brain by affecting the secretion of hormones and neurotransmitters. Similar to acupuncture, this approach activates opioid systems and has analgesic and anesthetic effects (10-11). Some acupoints (e.g. GB21, LI4, SP6, ST36, and BL32) are utilized to reduce pain intensity and improve labor process. The acupoints can stimulate uterine contractions and decrease labor pain by enhancing blood endorphin level, and consequently administer less oxytocin during labor (11-12).

Nowadays, other techniques such as TENS, laser, and electric currents are applied at the acupoints (13). TENS prevents pain transmission by activating descending inhibitory systems and increases blood flow near electrodes and consequently helps the healing or relaxation process of muscle spasm indirectly. TENS is non-invasive and safe, as well as causing a sense of self-control and self-efficacy in delivery experience. Additionally, applying TENS improves the quality of pushing in the second stage of labor because of saving mother's energy during labor and decreasing the anxiety and stress of mother (14, 15). Given the emphasis of World Health Organization (WHO) on applying non-pharmacological methods due to their simplicity, cheapness, safety, and more acceptability, the use of TENS at acupoints can relieve labor pain (16). Many studies have focused on the effect of TENS on labor pain, as

well as acupoints (17-18). However, some systematic review studies on the effect of TENS at lumbar and sacral regions on labor pain during 1997-2010 rejected the effectiveness of this technique in reducing the pain of labor and delivery (19-21). Shahoei et al. (2017) reported that applying TENS at lumbar and lumbosacral areas is associated with a significant decrease in the pain of labor and delivery (22). In a clinical trial, Aghamohammadi et al. (2011) used TENS at Hugo and Sanjiao acupoints simultaneously and reported a significant reduction in pain intensity during the first stage of labor in comparison with the TENS-placebo group (23).

Despite abundant advantages of TENS in relieving the pain of labor and delivery, the most effective point for electrode placement and electrical nerve stimulation is yet unknown. Therefore, the present study was conducted to compare the effect of TENS at the Hugo and Sanjiao acupoints on pain intensity during the first stage of labor in nulliparous women.

Materials and Methods

This randomized controlled clinical trial was conducted on 129 nulliparous mothers admitted to an Educational and Treatment Center in Zanjan, Iran for delivery during October 2022 to May 2023. The study was conducted based on CONSORT 2010 checklist. The nulliparous Iranian women aged 18-35 years and had $BMI < 30 \text{ kg/m}^2$ and height $> 150 \text{ cm}$ with gestational age of 38-40 weeks and educational level of fifth grade were included in the study. The other inclusion criteria were singleton pregnancy and cephalic presentation, as well as dilatation of 4-5 cm on arrival, spontaneous onset of uterine contractions, absence of cephalopelvic disproportion (CPD) according to the examination of the researcher and on-call physician, lack of membrane rupture more than six hours, no history of special disease or complication (epilepsy, heart diseases, skin complications or scar), and no addiction to tobacco and drugs, as well as not taking analgesics within three hours before intervention. The exclusion criteria were unwillingness to continue participating in the study, pregnancy termination by cesarean section for any reason, and burns or sensitivity at electrode position, as well as prescribing

prostaglandins, oxytocin, and other medications during labor.

The sample size was estimated based on the study by Aghamohammadi et al. (2011) (23). The calculation considered a standard deviation of 43 for pain duration or intensity across all three groups, a maximum significant difference (d) of 15 between the sample size and its actual value in the population, and three groups (k) in the study. The significance level (α) was set at 5%, and the test power ($1-\beta$) was 80%. This led to an initial sample size of 111 subjects. To account for a probable sample, drop of 10%, the final sample size was adjusted to 123 subjects.

At the beginning of the study, 129 participants were enrolled. However, 6 participants were excluded due to the need for emergency cesarean sections; finally 123 participants who remained in the study and were analyzed. The samples were then classified into three groups ($n = 41$ in each group) (Figure 1).

Convenience sampling was employed at first stage. The subjects were then classified by lottery using random sampling into Hugo (LI4), Sanjiao (SP6), and control groups. Each woman selected one of the three envelopes from a box, in which the name of the study group was written. For instance, if the written group name was Hugo, that participant was allocated to the Hugo intervention group. This method implemented one by one, ensuring a fair and random selection process for the sample, until the desired sample size was reached. To ensure an equal number of samples in each group, the sample counts were periodically checked, and adjustments were made as necessary.

A portable MAXTENS 2000 unit (Berries) with battery, two channels, and four pads (maximum power of 125) was applied. Before intervention (dilatation of 4-5 cm), pain intensity was measured using VAS in three groups. In both intervention groups, the device was set to continuous flow, 100 Hz/min, and 250 μ s (wavelength) and was alternately turned on for 20 min and turned off for 20 min until dilatation of 10 cm.

In the Hugo intervention group ($n=41$), two electrodes were placed in Hugo acupoints in both hands and in the Sanjiao intervention group ($n=41$), two electrodes were placed at Sanjiao acupoint in both feet. In the control group, in half

of them ($n=21,20$), two electrodes were placed in Hugo acupoints in both hands and in other half ($n=20$), two electrodes were placed at Sanjiao acupoint in both feet, however in this group, the TENS device was set off and was adjusted at the voltage of zero.

The researcher examined all the women vaginally in all stages (every 1-2 hours if needed). After the intervention, pain intensity was recorded using the VAS scale while the given dilatations (6 and 9 cm).

The data were collected using a questionnaire, labor management checklist, and VAS scale. The questionnaire contained 25 questions about demographic, as well as pregnancy and delivery-related data such as the age, educational level, job of mother, husband's job, household income, as well as gestational age, and attendance at childbirth preparation classes. Furthermore, the applied checklist included vaginal examination results, amniotic membrane status, the intensity of contractions, neonatal Apgar score, and possible side effects. Furthermore, the applied questionnaire and checklist were developed based on previous studies and relevant literature to ensure comprehensive data collection. Its content validity and reliability were confirmed through expert review and evaluation by specialists in the field of obstetrics and midwifery.

VAS is a visual scale to determine pain intensity, which is scored in the range of 0-10 so that 0-3, 4-7, and 8-10 indicate mild, moderate, and severe pain, respectively. Many studies have validated the scale and it is considered as one of the most widely used and valid pain measurement tools with proper reliability (24,25).

Descriptive statistics were utilized to present the numeric variables as mean \pm standard deviation (SD) or median (interquartile range [IQR]), while categorical factors were represented as frequency (percentage). The analysis of variance (ANOVA) or Kruskal-Wallis tests were employed to compare the mean or distribution of numeric variables across different levels of the group. Multiple comparisons were performed using Dunn-test correction. The Fisher's exact test was employed to assess the association between categorical variables and the groups. The impact of Hugo and Sanjiao compared to the control group at various dilation

levels was evaluated using the generalized estimation equation (GEE). All statistical analyses were performed using R (version 4.2.2). $P<0.05$ was considered statistically significant.

Results

The mean age of the participants was 23.78 ± 5.22 years, and no significant differences was found among the three groups ($P=0.792$). Similarly, the duration of marriage with mean of 3.45 ± 1.71 years was not significantly different among the groups ($P=0.080$). The mean

gestational age by LMP (39.43 ± 0.80 weeks) and gestational age determined by ultrasound (41.55 ± 30.04 weeks) did not differ significantly among the groups ($P=0.663$ and $P=0.640$, respectively). Regarding the decision to become pregnant, the majority of participants reported intentional and planned pregnancies (65.12%), followed by unplanned pregnancies (32.56%). Other variables were also reported in Table 1, showing no significant association among the groups (Table 1).

Table 1. Demographic characteristics of three groups

Variable	Total (N=129)	Group			P-Value
		Hugo (N=41)	Sanjiao(N=41)	Control (N=41)	
Age (years)	23.78 ± 5.22	24.24 ± 5.28	23.60 ± 5.17	23.53 ± 5.30	0.792
Duration of marriage (years)	3.45 ± 1.71	3.22 ± 1.72	3.91 ± 1.68	3.19 ± 1.68	0.080
Residence					
Urban	71 (55.04)	24 (58.54)	24 (53.33)	23 (53.49)	
Rural	58 (44.96)	17 (41.46)	21 (46.67)	20 (46.51)	0.880
Education					
Illiterate	2 (1.55)	0 (0.00)	1 (2.22)	1 (2.33)	
Undergraduate diploma	66 (51.16)	22 (53.66)	24 (53.33)	20 (46.51)	
Diploma	44 (34.11)	14 (34.15)	12 (26.67)	18 (41.86)	
Bachelor's degree	16 (12.40)	5 (12.20)	7 (15.56)	4 (9.30)	0.781
Master's degree	1 (0.78)	0 (0.00)	1 (2.22)	0 (0.00)	
PhD	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	
Spouse's education					
Illiterate	1 (0.78)	0 (0.00)	0 (0.00)	1 (2.33)	
Undergraduate diploma	54 (41.86)	19 (46.34)	18 (40.00)	17 (39.53)	
Diploma	52 (40.31)	16 (39.02)	18 (40.00)	18 (41.86)	
Bachelor's degree	21 (16.28)	6 (14.63)	8 (17.78)	7 (16.28)	0.990
Master's degree	1 (0.78)	0 (0.00)	1 (2.22)	0 (0.00)	
PhD	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	
Job					
Housewife	100 (77.52)	31 (75.61)	36 (80.00)	33 (76.74)	
Part-time employee	14 (10.85)	5 (12.20)	4 (8.89)	5 (11.63)	0.391
Employee	12 (9.30)	2 (4.88)	5 (11.11)	5 (11.63)	
Student	3 (2.33)	3 (7.32)	0 (0.00)	0 (0.00)	
Spouse's job					
Unemployed	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	
Self-employed	102 (79.07)	32 (78.05)	33 (73.33)	37 (86.05)	
Employee	26 (20.16)	8 (19.51)	12 (26.67)	6 (13.95)	0.336
Student	1 (0.78)	1 (2.44)	0 (0.00)	0 (0.00)	
The decision for pregnancy					
Involuntary	3 (2.33)	0 (0.00)	0 (0.00)	3 (6.98)	
Unplanned	42 (32.56)	14 (34.15)	14 (31.11)	14 (32.56)	0.323
Intentional and planned	84 (65.12)	27 (65.85)	31 (68.89)	26 (60.47)	
Income (million Tomans)					
<2	5 (3.88)	2 (4.88)	1 (2.22)	2 (4.65)	
3-6	85 (65.89)	25 (60.98)	33 (73.33)	27 (62.79)	0.784

Variable	Total (N=129)	Group			P-Value
		Hugo (N=41)	Sanjiao(N=41)	Control (N=41)	
6-9	37 (28.68)	14 (34.15)	10 (22.22)	13 (30.23)	
>9	2 (1.55)	0 (0.00)	1 (2.22)	1 (2.33)	
Gestational age by LMP (weeks)	39.43 ± 0.80	39.51 ± 0.68	39.36 ± 0.88	39.42 ± 0.82	0.663
Gestational age by ultrasound (weeks)	41.55 ± 30.04	38.80 ± 0.64	38.96 ± 0.77	38.93 ± 0.91	0.640

Numeric data were presented as mean ± standard deviation (SD), while categorical data were reported as frequency (percentage). The mean of numeric variables across different levels of the Group was compared using the analysis of variance (ANOVA). To assess the association between categorical variables and Group, the Fisher exact test was employed.

Prior to the intervention, the median pain score for the total sample was 7 (interquartile range [IQR]: 2-9). The median pain score for the Hugo group was 7 (IQR: 2-9), for the Sanjiao group was 3 (IQR: 2-9), and for the control group it was 8 (IQR: 2-9). However, no statistically significant differences were observed among the groups in pain scores ($P = 0.200$).

In terms of pain intensity at dilation 6 cm, the median score for the total sample was 4 (IQR: 2-7). The Hugo group had a median score of 3 (IQR: 2-5), the Sanjiao group had a median score of 3 (IQR: 2-6), and the control group had a median score of 7 (IQR: 3-8). The analysis revealed a statistically significant difference among the groups in terms of pain intensity ($P<0.001$). Post-hoc analysis using the Dunn-test correction

showed that both the Hugo and Sanjiao groups had significantly lower pain intensity compared to the control group.

Regarding to the pain intensity at dilation 9 cm, the median score for the total sample was 3 (IQR: 3-6). The median score for the Hugo group was 3 (IQR: 2-4), for the Sanjiao group was 3 (IQR: 3-6), and for the control group had a median score of 7 (IQR: 3-8). The analysis demonstrated a statistically significant difference among the groups in terms of pain intensity ($P<0.001$). Both the Hugo and Sanjiao groups demonstrated significantly lower pain intensity compared to the control group after post-hoc analysis (Table 2).

Table 2. Comparison of pain scores across dilation levels in three groups

Variable	Total (N=129)	Group			P-Value	Post Hoc
		Hugo (N=41)	Sanjiao (N=41)	Control (N=41)		
Pain intensity before intervention	7 (2, 9)	7 (2, 9)	3 (2, 8)	8 (2, 9)	0.200	-----
Pain intensity at 6 centimeters dilation	4 (2, 7)	3 (2, 5)	3 (2, 6)	7 (3, 8)	<0.001	Hugo < Control, Sanjiao < Control
Pain intensity at 9 centimeters dilation	3 (3, 6)	3 (2, 4)	3 (3, 6)	7 (3, 8)	<0.001	Hugo < Control, Sanjiao < Control

Note: The Kruskal-Wallis test was used to compare the distribution of numeric variables between different levels of Group. Multiple comparisons were performed using Dunn-test correction.

Table 3 and Figure 1-B presented the results of the analysis evaluating the effect of Hugo and Sanjiao interventions compared to the control during different dilation levels. At the baseline (pre-intervention), the comparison between Hugo and control showed no significant effect, with B coefficient of -1.13 (95% CI: -2.55, 0.30, $P = 0.121$). In addition, the comparison between

Sanjiao and control at the baseline indicated a significant lower B coefficient of -1.90 (95% CI: -3.18, -0.62, $P = 0.004$). Regarding the effect of dilation levels, the analysis revealed a non-significant association, with a B coefficient of -0.16 (95% CI: -0.35, 0.03, $P = 0.099$) at the control group. The interaction terms between Hugo vs. control and dilation, as well as Sanjiao vs. control

and dilation, were also examined. The interaction term [Hugo vs. control]*Dilation demonstrated a significant effect, indicating that the difference between Hugo and control varied across dilation levels, with a B coefficient of -0.99 (95% CI: -1.47,

-0.50, $p < 0.001$). Conversely, the interaction term [Sanjiao vs. control]* Dilation showed a non-significant effect, with a B coefficient of -0.17 (95% CI: -0.47, 0.12, $P = 0.252$) (Table 3) (Figure 2).

Table 3. Pain score during dilation levels in Hugo and Sanjiao intervention compared to control group

Parameter	B (95% CI)	P-Value
Hugo vs. Control	-1.13 (-2.55, 0.30)	0.121
Sanjiao vs. Control	-1.90 (-3.18, -0.62)	0.004
Dilation	-0.16 (-0.35, 0.03)	0.099
[Hugo vs. Control]*Dilation	-0.99 (-1.47, -0.50)	<0.001
[Sanjiao vs. Control]*Dilation	-0.17 (-0.47, 0.12)	0.252

Note: Dilation as within subject variables had three levels of before intervention, at 6 cm dilation, and at 9 cm dilation. The generalized estimation equation was used to evaluate the impact of Hugo and Sanjiao versus Control during levels of dilation.

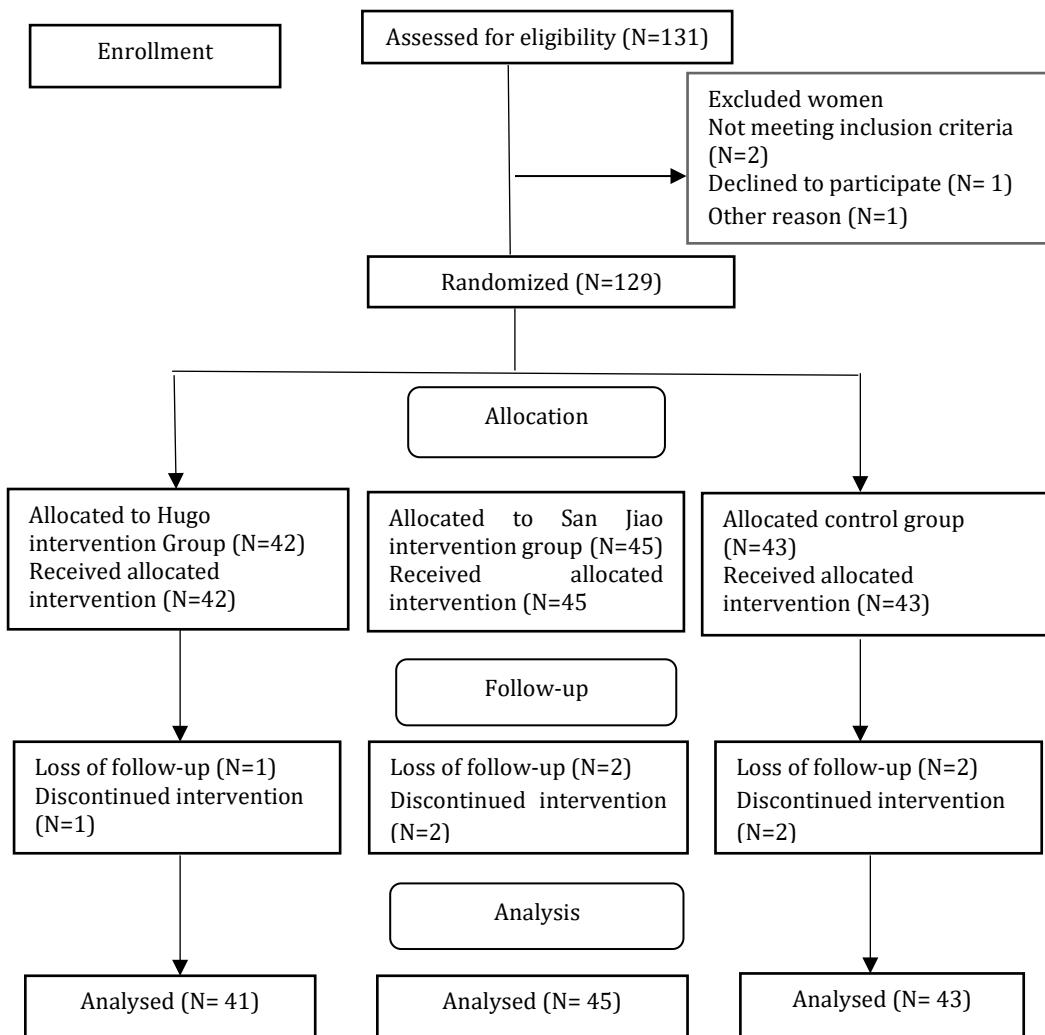


Figure 1. CONSORT Flowchart of the study

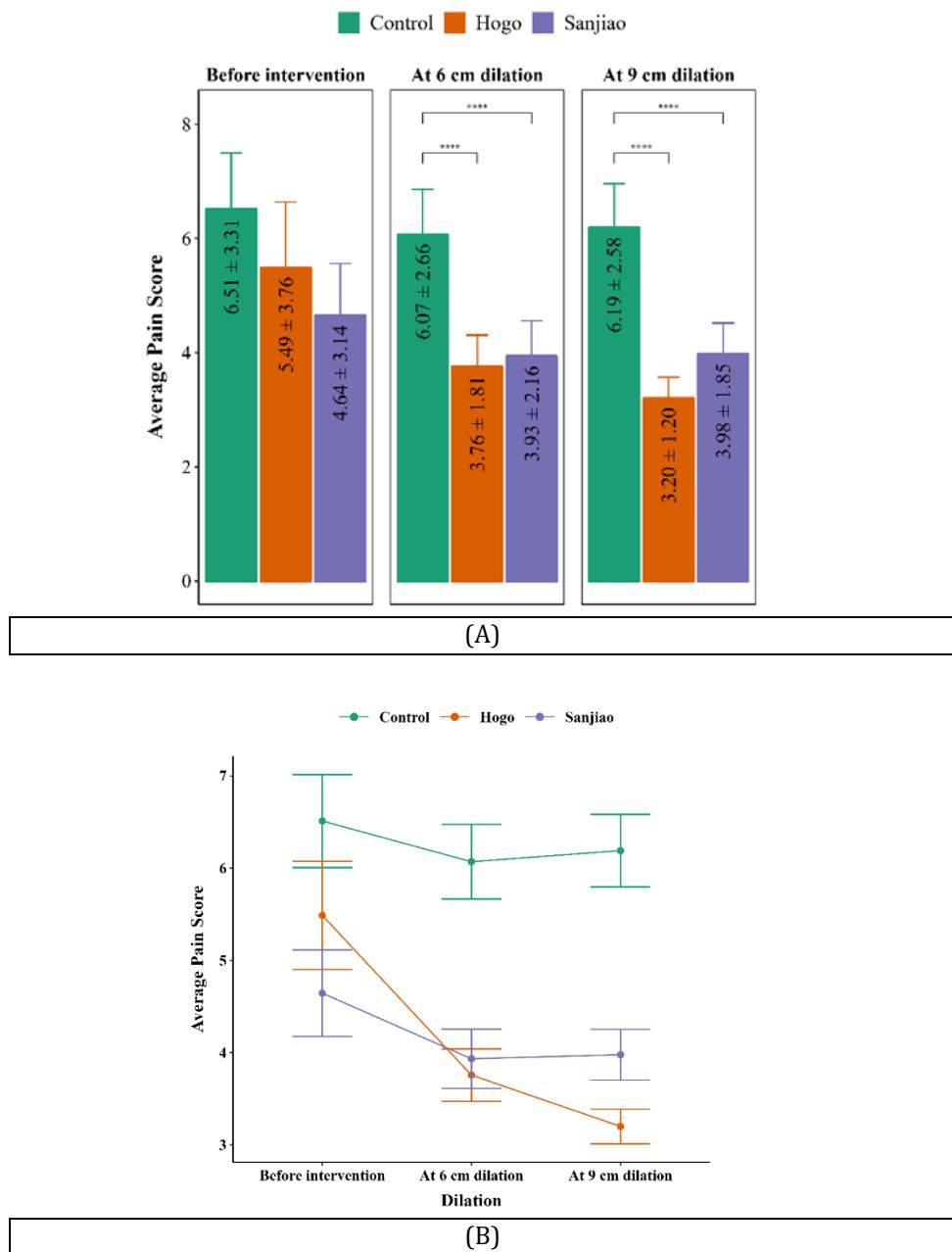


Figure 2. Variation in average pain score during dilation across three groups

Discussion

The purpose of the current research was to compare the effect of transcutaneous electrical nerve stimulation (TENS) at Hugo and Sanjiao acupoints on labor pain intensity during the first stage of labor in nulliparous women. As the

results of the present study revealed, applying TENS at Hugo and Sanjiao acupoints in dilatations of 6-7 cm and 9-10 cm led to a significantly decreased labor pain compared to the control group. However, Hugo and Sanjiao groups were not significantly different in terms

of pain intensity. Also, during the intervention, the results of GEE analysis showed a significantly decreased labor pain in the Hugo group compared to the control group. But this difference was not significant in the Sanjiao group and there was no significant difference between the two groups in terms of pain reduction.

Aghamohammadi et al. (2011) simultaneously applied four electrodes of TENS unit at Hugo and Sanjiao acupoints to reduce labor pain. They measured pain intensity by using VAS scale and reported that the mean pain intensity in the intervention and control groups in the dilatation of 6-7 cm was 7.5 and 8.34, respectively. The pain intensity was higher than the values obtained in the present study (mean pain intensity: 3 in the Hugo and Sanjiao groups). However, they found no significant difference between the intervention and control groups regarding labor pain (23), which is not consistent with the results of the present study. The difference between the study participants and delivery situation might arise this inconsistency.

Chao et al. (2007) focused on the effect of TENS simultaneously at Hugo and Sanjiao acupoints among 105 individuals (53 in the intervention and 52 in the control groups). The pain intensity was determined using visual analogue scale (VAS) and mean score of pain was 3 in the intervention group and 7.5 in the control group, which is in line with the results of the present study. Additionally, 96% of women in the intervention group decided to use this method in the next labor, while only 24% of those in the control group made such a decision (26).

Various researchers evaluated the effect of acupressure on the pain intensity of active phase. The results revealed a significantly lower pain intensity in different dilatations compared to the control group and suggested Hugo point as an efficient acupoint in decreasing labor pain (27, 28).

A systematic review on the effect of acupressure at the Sanjiao (SP6) and Hugo (LI4) acupoints on labor pain intensity reported that the pain intensity of the individuals treated with acupressure at the Sanjiao acupoint significantly reduced than the Hugo group (29). However, the results of the present study indicated no

significant difference between the two groups in terms of pain intensity.

Hanan et al. (2020) compared the effect of TENS at lumbar region with that of paracetamol infusion and pethidine intramuscular injection. The results demonstrated the greater effect of pethidine on labor pain relief compared to the TENS. Furthermore, TENS was significantly more effective in decreasing pain than paracetamol infusion. The education level and body mass index (BMI) were addressed as the factors related to the effectiveness of the assessed approaches. TENS was introduced as an optimal method to reduce labor pain because of the consequences of pethidine and paracetamol such as fetal asphyxia, need for caesarean section, and low neonatal Apgar score (30).

According to Karlinah and Irianti (2020), applying TENS at lumbar region in dilatation of 4-5 cm fails to relieve pain, while the women receiving TENS treatment in dilatation of 8 cm experienced significantly less pain. They declared that the early use of TENS may cause stress, as well as induce fear and pain (31). In the current research, the pain intensity relieved significantly in both 6 and 9 cm dilatation. The most noticeable reason of this difference between the present study and the study of Karlinah and Irianti is the different points which were used to attach TENS pads (lumbar and hugo-sanjiao).

The results of the systematic reviews on the effect of TENS at lumbar and sacral areas on labor pain revealed a significant difference between the placebo and TENS groups (32,33), which is inconsistent with the results of the present study. Researchers considered their results to be non-generalizable due to the methodological weakness in the performed clinical trials and difference in study design, as well as the ambiguity of the examined parameters. In addition, the studies have evaluated the effect of TENS at the lumbar and sacral regions (32, 33), while in the present study, the device was applied at Hugo and Sanjiao points.

The limitation of the present study is that labor pain intensity was measured based on the self-reporting of the subjects and there was no valid tool which can determine pain level. This limitation can be reduced to some extent by training the individuals regarding the

expectation of labor pain intensity. The other limitation of this study was related to the cases with an indication for cesarean delivery during the research, which were excluded from the intervention due to the impossibility to control this limitation by considering ethical considerations. Furthermore, this study focused on only nulliparous women. Thus, it is recommended to perform this technique among multiparous women in a comparative manner. This randomized controlled trial (RCT) followed CONSORT guidelines, ensuring methodological rigor and transparency. The carefully calculated sample size and objective pain assessment at multiple stages using the validated VAS scale enhance the reliability of findings. Additionally, the focus on specific acupoints (Hugo and Sanjiao) offers valuable evidence for TENS as a non-pharmacological pain management method during labor. Given that several studies have reported the significant effect of TENS on pain relief, it seems that it is useful and practical to conduct a wider study to compare the efficiency of the different points at which TENS electrodes are placed.

Conclusion

It seems that use of TENS at both Hugo and Sanjiao acupoints decreases labor pain intensity and no difference is observed between the points in terms of the effectiveness. Due to the safety and affordability of this method, it is recommended to apply it to reduce pain intensity to facilitate the labor process. It seems that this approach could increase mothers' satisfaction with the positive experience of vaginal childbirth; also could decrease the rate of labor and delivery complications as well as cesarean section. Also, it is important to train midwives to implement safe non-pharmacological interventions for labor process facilitation in order to provide all-round support to mothers during labor.

Declarations

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Conflicts of interest

The authors declared no conflicts of interest.

Ethical approval

All the participants signed the written informed consent to participate in the study. The study objectives, the optionality of participating in the study, as well as the assurance of confidentiality of data and the possibility of withdrawing from the study at any time during the study, were explained to the participants and written consent was obtained.

Code of Ethics

IR.ZUM.1398.0100.

Use of Artificial Intelligence (AI)

We acknowledge that no AI tools or technologies have been used to prepare this manuscript.

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Authors' contribution

MA: Study design, drafting of the manuscript and its critical revision for important intellectual content; ZKh: acquisition of data, writing the manuscript, revising manuscript; TShGh: Study design, critical revision of manuscript for important intellectual content, interpretation of data; RK: analysis of data, revising manuscript; SF: Study design, interpretation of data, acquisition of data, writing the manuscript.

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