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Original Research

The effect of transcutaneous electrical nerve stimulation (TENS) on pain control and phenylethanolamine-N-methyltransferase (PNMT) gene expression after cesarean section

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Abstract: Transcutaneous electrical nerve stimulation (TENS) is one of the non-pharmacological methods of pain relief that has been able to reduce pain by 70 to 90% in postoperative pain control. This study aimed to determine the effect of TENS on pain control after cesarean section and its effect on PNMT gene expression. For this purpose, a double-blind randomized clinical trial was performed on 70 Chinese patients with elective cesarean section. Patients were divided into case and control groups. In the case group, TENS and analgesic drugs were used to relieve pain, and in the control group, the only analgesic drug was used. Then the severity of pain, recurrence of pain attacks, the number of analgesic drugs used and the amount of analgesic drug used in the first 24 hours after surgery were evaluated and compared. Blood samples were also taken from patients to evaluate PNMT gene expression. The semi-quantitative RT-PCR was used to study changes in gene expression. The results showed that the group treated with TENS had less pain intensity and less recurrence of pain attacks than the group that received only analgesic medication. Also, the frequency of analgesic drug use and its dose in the TENS group were significantly lower than in the control group. TENS, on the other hand, has been able to greatly reduce the expression of the PNMT gene, which is produced during times of stress. Therefore, it is recommended that TENS be used as a non-invasive and non-pharmacological adjuvant effective in reducing pain after cesarean section.

Key words: TENS; Pain control; PNMT gene expression; Cesarean section.

Introduction

Recognition and treatment of pain are some of the oldest sciences that human beings have tried to reduce (1). Pain is caused by various factors and surgical incisions are one of the most common causes (2). Transcutaneous electrical nerve stimulation (TENS) is a non-pharmacological method of pain relief that is done by placing electrodes on the surface of the skin and electrical stimulation of nerves to relieve pain (3). The action mechanism of this device is not exactly known, but it activates a complex neuronal network to result in a reduction in pain (4). Hypothetically, this electrical current activates many afferent fibers that stimulate the inhibitory nerves of the posterior horn or release endorphins, or both (5). TENS also works by activating a descending inhibitory system to prevent pain transmission (3). It appears to inhibit the transmission of pain impulses through the A-delta and C fibers by stimulating the A-beta fibers (4). Biochemical mechanisms may also be involved, as TENS increases levels of substance P and 5-hydroxytryptamine in cerebrospinal fluid (6). On the other hand, stimulation may increase blood flow near the electrodes, which indirectly contributes to the healing process or relaxation of muscle spasms (7).

TENS has been very successful in controlling pain after surgery and its success rate is 70 to 90% (8). TENS pain control has been very useful after surgeries such as abdominal surgeries, thoracotomy, knee, laminectomy, urology, dental, and labor pains (9). Although there are no absolute limitations for TENS, it should not be used in heart patients using an artificial pacemaker or the elderly (10). It is also not recommended to place electrodes on the pregnant uterus, carotid sinus and eyeball (11).

The advantages of TENS include a non-invasive and safe way to control pain that nurses can easily use, and unlike other non-pharmacological methods of pain relief, using this method does not require much training (8, 11). Also, TENS has other benefits such as no side effects, non-toxicity, long-term use and ease of use (7). Researchers believe that using TENS is a strategy to reduce pain, but it cannot be a definitive cure for pain, and more research is needed (4, 6).

In the present study, we investigated the effect of TENS on pain intensity, recurrence of pain attacks, and frequency of analgesic drug use and analgesic drug dose in patients undergoing elective cesarean section. We also examined the expression of the phenylethanolamine-N-methyltransferase gene in these individuals and the control group. The product of this gene is produced during pain and stress to control stress levels by converting norepinephrine to epinephrine (12). Therefore, evaluating the expression of this gene, the role of TENS on

Table 1. Information of PNMT and TFRC primers.

Gene		Sequence	Temperature
PNMT	Forward	5′-AACCCGAACCTTCTGTCCTC-3′	86°C
	Reverse	5′-CAGAGTTAGACTGAACCCAGCTC-3′	
TFRC	Forward	3′-ACCGGCACCATCAAGCT-5′	87°C
	Reverse	3′- TGATCACGCCAGACTTTGC -5′	

the body's hormonal changes during pain can also be considered.

Materials and Methods

Patients

The present study was a randomized, double-blind clinical trial performed on 70 Chinese women who underwent elective cesarean section for the first time. General anesthesia was the same for everyone. According to the purpose of the study, alpha = 0.05%, beta = 20%, P1 = 40%, and P2 = 70%, the number of samples was estimated to be 35 for each group. Patients who were eligible to participate in the study were divided into case and control groups, respectively, by obtaining written and informed consent. All patients were examined and interviewed before the start of the study and the necessary explanations and training were given in the field of visual analogue scale (VAS) for pain. It should be noted that the VAS of pain, which was used as a tool to measure pain, was standard and was set in a 10 cm long chart with zero at the beginning and 10 at the end. No pain was measured with zero degrees and maximum pain was measured with ten degrees.

Anesthetics were the same for all and included Nesdonal 5 mg/kg, Scolin 1.5 mg/kg, Halothane 0.5%, Nitrose 50% and Oxygen 50%, Morphine 8mg, and Midazolam 2mg.

Immediately after the patient enters the recovery room and regains consciousness and their postoperative pain begins, after measuring the initial pain intensity based on a VAS, to relieve pain in the case group, TENS device (TENS 7000- 00092237616796) with a frequency of 100-150Hz for 30 minutes, and in the control group, analgesic drugs were used and again after 30 minutes, the pain intensity was measured with the same scale, which was done in the first 24 hours after surgery. It was performed every 6 hours. At the end of 24 hours, blood samples were taken from patients to measure gene expression. It should be noted that the electrodes of the TENS device were placed in pairs 5 cm above and below the operation site and the current intensity was adjusted according to the patient's needs and tolerance. Pain intensity, recurrence of pain attacks, number of analgesic drug use, and analgesic drug dose in both groups were accurately recorded in the first 24 hours after surgery. Also, before and after TENS and analgesic drug administration, patients' pulse, respiration, systolic and diastolic blood pressure were carefully measured and recorded.

RNA extraction and quantitative and qualitative measurement

After preparing a blood sample at the end of the first 24 hours of surgery, RNA was extracted from blood samples using the QIAamp RNA Blood Mini Kit by its protocol. The RNA precipitate was then dissolved in water treated with diethylpyrocarbonate and RNase inhibitor, and the extracted RNA was stored at -80 °C. In order to evaluate the quality of the extracted total RNA, RNA samples were electrophoresed on 1% agarose gel and the clear bands of 28s and 18s ribosomal RNAs and the approximate ratio of two to one band were considered as criteria for the integrity of the extracted total RNA. Quantitative measurements were performed by spectrophotometry to determine the total RNA concentration and the absorbance obtained at 260 nm was used to determine the total RNA concentration extracted from the samples.

Evaluation of changes in PNMT gene expression

The semi-quantitative RT-PCR was used to study changes in gene expression. The mRNA sequence of PNMT genes was obtained from the relevant database data. To prevent possible replication of DNA, the reverse primer was designed for the genes in the binding regions of the two exons. TFRC was used as the housekeeping gene. The sequences of primers are shown in Table 1. In addition, to ensure the possible non-duplication of genomic DNA fragments during PCR, NRT control (No reverse transcriptase control) and NTC control (No template control) were used. Due to the lack of amplification of the expected fragments and other fragments in PCR, these control samples did not use DNase treatment on the extracted RNAs.

Statistical analysis

The data of each group were calculated as Mean ± SEM and comparisons and statistical analysis of these data were performed using t-student test and SPSS statistical software (IBM SPSS Statistics 22.0). In all statistical comparisons, the level of significance $P < 0.05$ was considered.

Results

Clinical effect of TENS

The results showed that 47 (73%) of the patients were in the age group of 20-35 years. Pain intensity, measured by TENS, was between 6 to 7 in 58% of patients of the case group, and was between 7.1 to 8 in 57% of the control group, so that the mean pain intensity was 5.9±0.8 and 7.9±0.5 in the case and control groups, respectively. T student test showed a significant difference ($P<0.001$) (Table 2).

Pain recurrence attacks were between one to two times in 40% of patients, in the case group, and between five to six times in 57% of patients, in the control group. The mean pain recurrence attacks were 1.78±1.3 times and 6.03±1.15 times in the case and control groups, respectively, which showed a significant difference ($P<0.001$) (Table 3).

Table 2. Frequency distribution of patients according to pain intensity in case group (after using TENS) and control group (after analgesic drug use).

Pain Intensity	Case Group (number)	Control Group (number)
<6	15 (42%)	0 (0%)
6-7	20 (58%)	4 (11%)
7.1-8	0 (0%)	20 (57%)
8.1-9	0 (0%)	11 (32%)
9.1-10	0 (0%)	0 (0%)
Total	35 (100%)	35(100%)

Table 3. Frequency distribution of patients according to pain recurrence attacks in case group (after using TENS) and control group (after analgesic drug use).

Pain Recurrence Attacks	Case Group (number)	Control Group (number)
0	10 (28%)	0 (0%)
1-2	14 (40%)	0 (0%)
3-4	11 (32%)	4 (11%)
5-6	0 (0%)	20 (57%)
7-8	0 (0%)	11 (32%)
Total	35 (100%)	35 (100%)

37% of the patients in the case group did not use any analgesic drugs after using TENS, while 40% of the patients in the control group used analgesic drugs 4 times. Therefore, in the case and control groups, the average number of analgesic drugs consumed was 0.94±0.93 and 9.4±1.03, respectively. The student's t-test showed a significant difference in this regard (P<0.001) (Table 4).

The results also showed that, in the first 24 hours after surgery, the mean amount of analgesic in the case and control groups was 47.4±46.9 mg and 204.7±51.4 mg, respectively. Therefore, they showed a very significant difference (P<0.001).

PNMT gene expression

The gel electrophoresis image of the PNMT gene and TFRC gene expression is shown in Figure 1. The intensity of the obtained bands was converted to quantitative values, and the quantitative values obtained for the expression of the PNMT gene, in each individual, became normal compared to TFRC. The results of the quantification of gene expression bands, in both experimental groups, are shown in Figure 2. The results showed that the expression of the PNMT gene in the group with electrical stimulation (24±2.1%) had a significant decrease compared to the analgesia drug consumer group (76±9%) (P<0.001).

Table 4. Frequency distribution of patients according to number of analgesic drugs usage in case group (after using TENS) and control group (after analgesic drug use).

number of analgesic drugs usage	Case Group (number)	Control Group (number)
0	13 (37%)	0 (0%)
1	11 (32%)	1 (3%)
2	8 (23%)	1 (3%)
3	3 (8%)	8 (23%)
4	0 (0%)	14 (40%)
5	0 (0%)	9 (26%)
6	0 (0%)	2 (5%)
Total	35 (100%)	35 (100%)

Discussion

The findings of this study indicate that the mean pain intensity after surgery was equal to 5.9±0.8 in the case group (TENS group), and it was equal to 7.9±0.5 in the

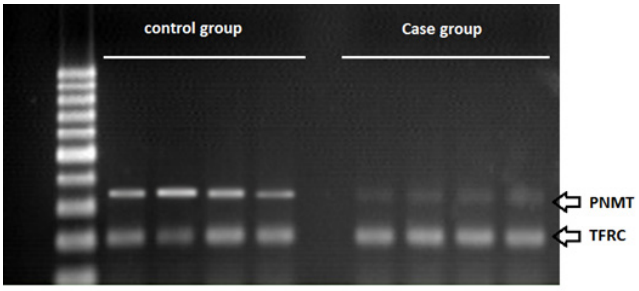

Figure 1. Image of gel electrophoresis related to PNMT and TFRC gene expression in control groups (analgesia drug consumer group) and case group (TENS group).

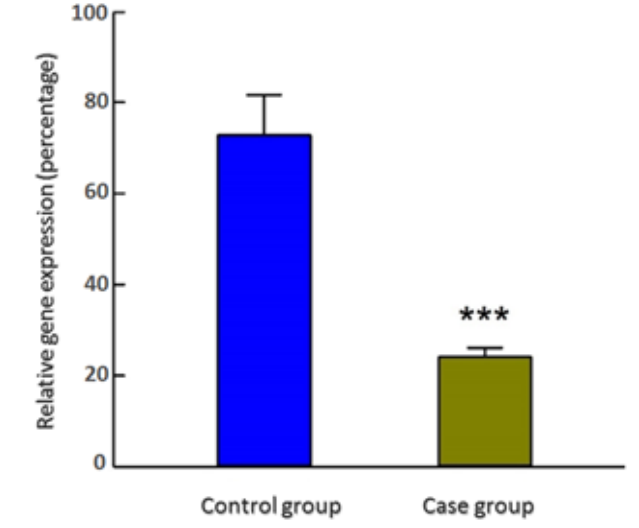

Figure 2. Comparison of relative changes in PNMT gene expression in control groups (analgesia drug consumer group) and case group (TENS group).

control group (analgesia group). Therefore, patients had the lowest pain intensity when using TENS and analgesic drugs, and the highest pain intensity happened when using the analgesic drugs alone. This difference between the mean pain intensity was significant in the two groups (P<0.001). A study by Kayman-Kose *et al*. on the effect of TENS on reducing pain after cesarean section showed that TENS significantly reduced analgesic drug use by up to 50%, as well as the severity and duration of pain in the case group (13). Other studies have also generally shown the effect of TENS in reducing pain by up to 70% compared to not using it (14-16).

Regarding the mean recurrence of pain attacks, it was 1.78±1.3 times in the case group and 6.03±1.15 times in the control group. Therefore, patients who used TENS had the lowest number of pain attacks in the first 24 hours after surgery, which was significant in comparison to the control group (P<0.001).

In a study conducted by Fatima and Sarfraz, on the use of TENS for analgesia after cesarean section, the results showed that the case group was evaluated as good to excellent in terms of analgesia and it had the lowest consuming number of analgesics (17).

Findings of the average number of analgesics consumed were 0.94±0.93 times in the case group, and 4.09±1.03 times in the control group. The mean dose of the analgesic drug was 47.4±46.9 mg in the case group, and 204.7±51.4 mg in the control group. As a result, the consuming number of analgesics and the consuming dose of analgesics in the case and control groups show a significant difference (P<0.001).

There have been many studies that compare the effects of TENS and the use of analgesics on pain relief after surgery. For example, a study on the effects of TENS on lower abdominal surgery showed that the use of TENS significantly reduced the need for analgesics and did not have the side effects of analgesics drugs (18).

Also, the results of the present study showed that TENS was able to significantly reduce the expression of the PNMT gene in the case group compared to the control group. The product of the PNMT gene is an enzyme called phenylethanolamine-N-methyltransferase, which is found naturally in the adrenal gland (19). This enzyme converts norepinephrine to epinephrine (12). When the body is under stress, epinephrine increases heart rate, constricts arteries, and dilates the airways, and is involved in the sympathetic nervous system's response to war and flight (20-24). Therefore, it can be concluded that TENS can reduce stress by reducing the expression of the PNMT gene and thus help reduce pain.

Conclusion

The present study was performed to evaluate the effect of transcutaneous electrical nerve stimulation (TENS) on pain relief after cesarean section and its role in phenylethanolamine-N-methyltransferase (PNMT) gene expression. The results showed that the group treated with TENS had less pain intensity and less recurrence of pain attacks than the group that received only analgesic medication. Also, the frequency of analgesic drug use and its dose in the TENS group were significantly lower than in the control group. TENS, on the other hand, has been able to greatly reduce the expression of the PNMT gene, which is produced during times of stress. Therefore, it is recommended that TENS be used as a non-invasive and non-pharmacological adjuvant effective in reducing pain after cesarean section.

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