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Effect of Electrical Stimulation Followed by Exercises in Postnatal Diastasis Recti Abdominis via MMP2 Gene Expression

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ABSTRACT

Since abdominal muscle training is one of the most important ways to treat rectal diastasis, it is necessary to design and provide appropriate exercises to treat this problem. One of the complementary methods to achieve higher intensity training is the use of electrical muscle stimulation along with voluntary activity, which causes maximum recall in muscle units. Therefore, in the current study, the effect of electrical stimulation followed by exercises was evaluated in postnatal diastasis recti abdominis via MMP2 gene expression. For this purpose, we studied on thirty-two women who had rectal diastasis for six months and were referred to a physiotherapy clinic by a gynecologist. They were divided into the control group (n=16) and the intervention group (n=16). The distance between the two blocks of the rectus abdominis muscle and the thickness of the abdominal muscles at rest was determined by ultrasound. In the intervention group, electrical stimulation and strengthening exercises of oblique muscles were performed for six weeks. The control group did not perform any specific exercises. After six weeks, another ultrasound was performed. The expression of the MMP2 gene was measured by the real-time PCR method. Comparison of the distance between the two blocks of rectus abdominis muscle (above the umbilicus and below the umbilicus) before and after six weeks showed that in the experimental group after intervention in both areas had a significant decrease (above the umbilicus = 0.001 and below the umbilicus P = 0.03), while this distance in the control group did not decrease significantly (p >0.05). Also, in the upper part of the umbilicus, the distance between the two rectus abdominis muscle blocks in two groups after the intervention showed a significant difference (p = 0.04). Evaluation of MMP2 gene expression showed that there was no significant difference between the two groups before the intervention (p >0.05). However, after the intervention, the expression of this gene decreased significantly in the intervention group (p = 0.007). In general, the present study results showed that electrical stimulation of abdominal muscles with strengthening exercises of internal and external oblique muscles could reduce rectal diastasis and increase the thickness of these muscles in people with rectal diastasis.

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Introduction

Increasing the distance between the two blocks of the rectus abdominis muscle begins from the fourteenth week of pregnancy and continues until delivery (1). The most significant improvement in the distance between the two blocks of the right abdominal muscles occurs up to the eighth week after delivery. It reaches a stable level in 6 months after delivery (2). In many women, rectal diastasis does not improve even months or years after delivery. Various reports of diastasis occur during the third trimester of pregnancy, ranging from 66 to 100% (3). The prevalence of rectal diastasis six months after delivery was reported to be 39-35%, with the highest majority immediately after delivery in women who had multiple deliveries, about 98% (1).

Abdominal wall muscles play an essential role in body alignment, trunk and pelvic stability, breathing, trunk movements, and abdominal support (4). Increasing the distance between the two muscles of the right abdomen threatens these activities. Weakness of the anterior abdominal wall muscles can lead to changes in body orientation and increased mobility in the lumbar-pelvic area; therefore, the person becomes prone to stretching and damage to the sacral region and the sacrum (5). Weakness in the central muscles

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leads to weak force in the sacroiliac joint, which leads to instability in the pelvis (4). This factor causes back and hips joint pain. In addition, abdominal activities such as breathing, coughing, urination, defecation, childbirth, and singing are affected in rectal diastasis. An abdominal hernia is another complication of rectal diastasis (6).

Rectal diastasis is usually not painful and is often overlooked. Due to the vital role of abdominal muscles in providing the lumbar and pelvic region stability, correction of rectal diastasis in people seems necessary (7). Various methods have been reported to treat rectal diastasis, one of which is surgery. Surgical correction of rectal diastasis is a controversial procedure because it has many side effects: blood clot formation, wound infection, skin graft loss, prominent wound effect, increased abdominal pressure, decreased venous return, and increased risk of blood clots in deep veins (8). In 40% of cases, diastase recurrence has been reported. Another way to correct rectal diastasis is abdominal muscle exercises after pregnancy, but the effect of abdominal wall exercises on diastasis is not yet apparent (9). On the other hand, electrical stimulation of muscles can be introduced as an effective method to correct rectal diastasis. Electrical Muscle Stimulation creates muscle contraction by creating internal electrical stimulation, which is more than a transient process and is a new method of forming muscle or losing weight that can make the muscles more intense throughout the exercise (10).

Functional changes and morphological features of skeletal muscle occur under different conditions and in response to a wide range of pathological and physiological stimuli, most of which are dependent on extracellular matrix (ECM) changes (11). The overexpression of many genes in response to exercise is closely related to the extracellular matrix. Hence, different sports exercises make many changes in the expression of genes that regulate the structure of the extracellular matrix, myofibrils, and membrane protein compounds. As a result, metabolic enzymes improve the efficiency of skeletal muscle function in new sports activities (12). Matrix metalloproteinases (MMPs) regulate cellular matrix compounds and are known as zinc and calcium-dependent endopeptidases to induce changes in the extracellular matrix structure (13). The human genome has 24 matrixin genes. Each metalloproteinase matrix targets a specific substrate, so appropriate MMPs are released at specific times and places in response to certain behaviors to modulate membrane changes and compatibility (14). The role of MMPs in skeletal muscle is to cooperate in matrix degradation while the release of MMPs facilitates angiogenesis in the bloodstream. Matrix metalloproteinase-2 (MMP2) is considered the most critical MMPs related to skeletal muscle function or dysfunction (15).

This study aimed to evaluate the effect of electrical stimulation followed by exercises in postnatal diastasis recti abdominis via MMP2 gene expression.

Materials and methods

Study population

This study was a randomized clinical trial with a control group. The study population was women who had rectal diastasis for six months and were referred to a physiotherapy clinic by a gynecologist. After obtaining informed written consent, they entered the study and were randomly divided into two intervention and control groups. Inclusion criteria include the distance between two blocks of right abdominal muscle more than 2 cm, at least six months after delivery, age between 18-45 years, no history of abdominal hernia and abdominal surgery, no chronic low back pain, no pain in the back, neck, and thorax, and no abdominal exercises for the past six months. People should not have done activities that lead to fatigue before the assessment sessions. If the person did not want to continue practicing at any study stage, people were excluded from the study. A total of 32 women with rectal diastasis were included in the study. They were divided into control (16 cases) and intervention (16 cases) groups.

Diagnosis and evaluation of rectal diastase

First, the presence or absence of rectal diastasis was assessed by finger measurement. For this purpose, the participant lay on the bed, a pillow under the head, and the knees bent 90 degrees; in this way, the examiner placed index and middle fingers slightly above the person's navel in a horizontal position, and the person was asked to lift her head off the ground. If the fingers were sunk, the anterior abdominal wall was felt on either side of the examiner's fingers, and the distance was two fingers or more, rectal diastasis was diagnosed. Their rectal diastasis was then confirmed by ultrasound.

Ultrasound was first performed while the supine person was laying on the bed, with a pillow 90 degrees below the head and knees, and the soles of the feet on the floor and hands on the side of the body. An ultrasound probe with a frequency between 5-10 Hz was used. A wide range of frequencies is used to observe the muscles, but a higher frequency with a linear probe gives better images (16, 17). In this study, 7.5 MHz core frequency and type B current were used. The method used in previous studies and its reproducibility and reliability for measuring rectal diastasis was used to measure the distance between two right abdominal muscle blocks (18-20). Although the most significant distance between the two blocks of the right abdominal muscle is at the level of the umbilicus, due to technical difficulties, measurements were not made at the level of the umbilicus (18, 21), but above and below the umbilicus; First, to standardize the location of the probe, a line was magically drawn just 2 cm above the umbilicus and below the umbilicus, then the lower edge of the probe was placed horizontally on the marked line. When the tester could see the two edges of the right abdominal muscle on the device screen by moving the probe left and right without pressing the probe into the abdomen, the image was recorded when the sample was exhaled.

An ultrasound assessed the internal oblique, external oblique, abdominal transverse and right abdominal muscles before and after six weeks at rest in all samples. The image took at the end of the exhaled phase to measure muscle thickness, and the measurement lines were perpendicular to the fascia separating the muscles.

Exercise and electrical stimulation to strengthen internal and external muscles

In the intervention group (n = 16), after installing the electrodes of the electrical muscle stimulation device, strengthening exercises were given for internal and external oblique muscles; To contract the muscle of the right internal inclination and the left external inclination, the person bends over with the knees bent while the soles of the feet are on the ground, leaning the head and torso clockwise until the lower edge of the scapula rises. She lifted the ground and reached the tips of her fingers to her right knee. To contract the left internal and right external oblique muscles, the person is bent over with the knees bent while the soles of the feet are on the ground, with the head and torso tilted counterclockwise until the lower edge of the scapula rises off the ground. Reaching the fingertips of the hand raised the left knee (22). Individuals were asked to perform the exercises once a day for six weeks each day, with each exercise lasting five seconds and resting for 10 seconds; and repeating 20 times for each.

Before electrical stimulation to reduce skin resistance, the area was cleansed with alcohol and cotton wool, and electrodes were placed on both rectus abdominal muscles. The active electrodes of each canal were located at 3 cm from each other. It was set and fixed entirely with a cloth band. The intensity of the current increases to the point that the maximum tolerable contraction. No treatment was performed in the control group.

Evaluating MMP2 gene expression

At the beginning of the exercise period, 10ml of the left arm vein blood samples were taken from all participants after 11 hours of fasting in the morning. The second stage of blood sampling was performed at the end of the sixth week, 48 hours after the last exercise session, and the samples were frozen at -80°C. RNA extraction and cDNA synthesis were obtained by innuPREP Blood RNA Kit (Analytik Jena GmbH) and RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA), respectively. The relative mRNA level of the MMP2 gene was measured using Real-time PCR (Smart cycler, USA) technique. This method was performed with the help of MMP2 specific primer, and β-actin specific primer was used to control the proliferation of this gene (Table 1). The real-time PCR program included 50°C for 2 minutes, 95°C for 10 minutes, 95°C for 30 seconds, and 60°C for 40 seconds (two final stages of 35 cycle repetition). The formula $2^{-\Delta\Delta CT}$ was used to quantify the expression values of the target gene.

Table 1. Primer sequences for MMP2 gene and β-actin gene

Gene		Primer Sequence
MMP2	Forward	5'- GTGCCATGTAAATCCCCACT-3'
	Reverse	5'-CTCCACTCCTCCCTTTCCTC-3'
β-actin	Forward	5'-ACCGAGCGCGGCTACAG-3'
	Reverse	5'-CTTAATGTCACGCACGATTTCC-3'

Statistical analysis

To describe the present study's data, descriptive statistics, including mean and standard deviation, were used. Kolmogorov-Smirnov test was used to determine the normal distribution of data. An independent t-test was used to compare the two control and intervention groups. A paired T-test was used to compare the MMP2 gene expression within the group. The present study data were analyzed using SPSS software version 22 at a significance level of $P \leq 0.05$.

Results and discussion

Demographic characteristics of patients

A total of 32 people were eligible for the study, of which 16 were in the intervention group and 16 were in the control group. Table 2 lists the demographic characteristics of patients in test and control groups. Based on the results, participants in the two groups were identical in terms of demographic characteristics. There was no significant difference between the general characteristics of the intervention and control groups ($p > 0.05$).

Table 2. Comparison of patients' demographic characteristics in the intervention and control groups

Variable	Intervention group (Mean ± SD)	Control group (Mean ± SD)	P-value
Age (year)	33.47 ± 0.3	35.1± 4.2	0.1
Weight (kg)	69.3 ± 5.1	68.06 ± 3.8	0.6
Height (cm)	168.6 ± 3.7	165.1 ± 4.6	0.07
Body mass index (kg/m^2)	25.1 ± 2.1	24.7 ± 1.6	0.2
Number of children	1.53 ± 0.61	1.55 ± 0.59	0.8

The distance between the two blocks of the rectus abdominis muscle and the thickness of the abdominal muscles

The comparison of the mean distance between two blocks of the rectus abdominis muscle and thickness of the abdominal muscles before and after 6 weeks and also between the intervention and control groups in women with rectal diastasis is shown in Table 3. Comparison of the distance between the two blocks of rectus abdominis muscle (above the umbilicus and below the umbilicus) before and after six weeks showed that in the experimental group after six weeks of intervention in both areas had a significant decrease (above the umbilicus = 0.001 and below the umbilicus P = 0.03), while this distance in the control group did not decrease significantly in any of the measurements above and below the umbilicus ($p > 0.05$).

Table 3. Comparison of the mean distance between two blocks of the rectus abdominis muscle and the thickness of the abdominal muscles between the maxillary and control groups

Variable	Group	Before intervention	After intervention	P-value
Distance between the two blocks of the rectus abdominis muscle (above umbilicus) (cm)	Intervention	3.1 ± 0.7	2.4 ± 0.7	0.001
	Control	2.9 ± 0.6	2.9 ± 0.7	0.7
	P-value	0.5	0.04	
Distance between the two blocks of the rectus abdominis muscle (under umbilicus) (cm)	Intervention	2 ± 0.6	1.7 ± 0.6	0.03
	Control	1.8 ± 0.7	1.4 ± 0.4	0.1
	P-value	0.5	0.1	
Thickness of rectus abdominis muscle (right side) (cm)	Intervention	0.8 ± 0.1	0.8 ± 0.09	0.5
	Control	0.7 ± 0.1	0.7 ± 0.7	1
	P-value	0.5	0.5	
Thickness of rectus abdominis muscle (left side) (cm)	Intervention	0.8 ± 0.1	0.7 ± 0.1	0.8
	Control	0.7 ± 0.1	0.7 ± 0.1	1
	P-value	0.3	0.7	
External oblique muscle thickness (right side) (cm)	Intervention	0.4 ± 0.06	0.5 ± 0.08	0.001
	Control	0.4 ± 0.08	0.4 ± 0.08	1
	P-value	0.9	0.01	
External oblique muscle thickness (left side) (cm)	Intervention	0.4 ± 0.06	0.5 ± 0.07	0.002
	Control	0.4 ± 0.08	0.4 ± 0.06	0.6
	P-value	0.5	0.01	
Internal oblique muscle thickness (right side) (cm)	Intervention	0.5 ± 0.1	0.6 ± 0.1	0.001
	Control	0.6 ± 0.09	0.6 ± 0.08	0.8
	P-value	0.2	0.01	
Internal oblique muscle thickness (left side) (cm)	Intervention	0.5 ± 0.09	0.6 ± 0.1	0.01
	Control	0.6 ± 0.1	0.6 ± 0.1	1
	P-value	0.1	0.02	
Thickness of the transverse abdominal muscle (right side) (cm)	Intervention	0.4 ± 0.06	0.4 ± 0.07	0.8
	Control	0.4 ± 0.07	0.4 ± 0.06	0.8
	P-value	0.08	0.08	
Thickness of the transverse abdominal muscle (left side) (cm)	Intervention	0.4 ± 0.03	0.4 ± 0.05	0.7
	Control	0.4 ± 0.07	0.4 ± 0.07	1
	P-value	0.1	0.05	

Also, in the upper part of the umbilicus, the distance between the two rectus abdominis muscle blocks in the experimental group and the control group after the intervention showed a significant difference (p =

0.04). This difference between the two groups was not significant after the intervention (p >0.05).

Evaluating MMP2 Gene Expression

Evaluation of MMP2 gene expression showed that there was no significant difference between the two groups before the intervention (p >0.05). However, after the intervention, the expression of this gene decreased significantly in the intervention group (Figure 1) (p = 0.007).

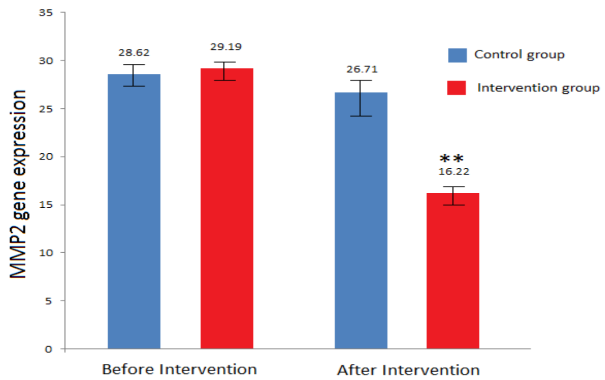

Figure 1. The expression of MMP2 gene expression before and after intervention between control and intervention groups; **: P<0.01

Since abdominal muscle training is one of the most important ways to treat rectal diastasis, it is necessary to design and provide appropriate exercises to treat this problem (23). One of the complementary methods to achieve higher intensity training is the use of electrical muscle stimulation along with voluntary activity, which causes maximum recall in muscle units (24). Given that different abdominal wall muscles can affect the distance between the rectus abdominis muscles, it is necessary to determine the effect of strengthening the training of other groups of these muscles in the treatment of rectal diastasis (25).

This study aimed to investigate the effect of strengthening exercises of internal and external oblique muscles on the distance between two rectus abdominis muscles and the thickness of different abdominal wall muscles in women with rectal diastasis. Comparing the distance between the two rectus abdominis muscle blocks before and after six weeks of training in the intervention group, the distance between the two rectus abdominis muscles above and below the umbilicus was significantly reduced. This distance increased from 3.12 to 2.40 cm in the upper part of the umbilicus and 2 to 1.74 cm in the lower parts. While in the lower umbilical region, this distance was not significantly different between the two groups after the intervention.

Evaluation of MMP2 gene expression showed that there was no significant difference between the two groups before the intervention (p >0.05). However, after the intervention, the expression of this gene decreased significantly in the intervention group (p = 0.007). Hadler- Olsen *et al.* Examined glycolytic activity and MMP2 in the control and case groups, which was consistent with the present study's findings (26). They attributed the decline in MMP2 activity to the fact that if the contractile process stimulates MMP-2 intracellular activity, it could be a controlling mechanism to prevent overexpression of this enzyme in skeletal muscle or increase the extracellular need for MMP2.

In another study, the expression of this gene was not significantly different between the control and intervention groups (27). The possible reason for the lack of significant difference in MMP2 between the two groups is the low involvement of tense-contraction muscle fibers in the training program because glycolytic enzymes are found immediately in type II muscles and are suppressed by M2. Therefore, MMP2 mRNA and protein are more expressed in type II muscle fibers. One of the reasons for the inconsistency of this study with the present study is the intensity of the exercises used and the gender differences of the subjects.

In general, the present study results showed that electrical stimulation of abdominal muscles with strengthening exercises of internal and external oblique muscles could reduce rectal diastasis and increase the thickness of these muscles in people with rectal diastasis.

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Interest conflict

None.

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