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Research Article

The effect of exercise and vitamin B6 on the expression of COX2 and IL-1B genes in endometrial tissue of endometriosis rats

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Abstract

Background: Endometriosis is a chronic disease, afflicting about 5-10% of women. Many possible environmental and molecular factors have been proposed for the pathogenesis of endometriosis; nevertheless, its real mechanism is still under investigation. This research aimed to examine the effect of physical exercise and B6 vitamin on the expression of COX2 and IL-1B genes on endometriotic tissue in endometriosis model rats.

Materials and Methods: In this experimental research, 25 Wistar rats were randomly assigned into five groups: control-healthy, endometriosis, endometriosis + exercise, endometriosis + B6, endometriosis + exercise + B6. Vitamin B6 were administered as 60 mg/kg per body weight of each rat. The swimming exercise program involved 8 weeks of exercise, each week 5 days, and each day for 30 min. For data analysis, one-way analysis of variance (ANOVA) and post hoc Tukey test were used.

Results: The extent of expression of COX and IL-1β genes increased significantly in the endometriosis group compared to the control-healthy group. All three groups of endometriosis + exercise, endometriosis + B6, and endometriosis + exercise + B6 showed a significant reduction of COX2 expression compared to the endometriosis group. Two groups of endometriosis + exercise and endometriosis + exercise + B6 indicated a significant decline in the extent of expression of 1L-1β gene, as compared to the endometriosis group. The endometriosis + exercise + B6 group revealed a significant reduction in the extent of expression of COX2 and IL-1β genes compared to endometriosis + B6. Finally, the endometriosis + exercise group revealed a significant decrease in the extent of expression of IL-1β gene, when compared with the endometriosis + B6.

Conclusion: Overall, the results suggest that changes in the key molecules or signaling pathways as well as gene expression in the endometriosis process can contribute to improving this condition. Doing physical activity and concurrent consumption of B6 vitamin will be helpful in curbing this disease and improving the level of this condition.

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1. Introduction

Endometriosis is a chronic disease afflicting around 5-10% of women (1). Endometriotic tissues emerge and grow in extrauterine region especially the pelvis (2). Endometriosis is often found in organs including cervix, ovaries, vagina, intestine, posterior cul-de-sac, uterine ligament, pelvic peritoneum and rectovaginal septum, urinary system, abdominal wall, chest cavity, lungs, and central nervous system (3). Although a large number of patients with endometriosis are asymptomatic, it may be associated with menstruation pain, painful intercourse, and chronic pelvic pain (4). Many possible environmental and molecular factors have been proposed for the pathogenies of endometriosis, but is real mechanism is still under investigation (5). Cyclooxygenase enzyme-2 (COX-2) converts arachidonic acid to prostaglandin H2, which is a precursor of different molecules including thromboxanes, prostacyclins, and prostaglandins. COX-2 enzyme is usually expressed only in inflammatory cells. COX-2 can be expressed in response to various stimuli such as hormones, mitogens, cytokines, inflammatory mediators, and growth factors. It is believed that COX-2 is involved in carcinogenesis through promotion of angiogenesis, increased cell attack, inhibition of apoptosis, and stimulating cell proliferation (6). Overexpression of COX-2 has been proven as a major regulator in the progression of endometriosis (7). IL-1β cytokines are produced out of macrophages and monocytes. IL-1β is another important cytokine, involved actively in inflammatory responses in humans. In endometriosis, peritoneal macrophages of women with this disease produce higher levels of IL-1β. IL-1β stimulates endometrial cells for secretion and production of cytokines plus growth factors, and plays a key role in binding, growth, and angiogenesis of endometriotic tissue (8).

Proinflammatory cytokine, IL-1β, is an established factor which regulates expression of COX-2 in patients with endometriosis. COX-2 gene is more sensitive to eutopic cell and to stimulation of IL-1β in extrauterine endometriotic stroma cells. These results indicate that inflammation plays a role in some pathogenic aspects of endometriosis (9). Various treatments have been considered for endometriosis; it has been shown that these treatments affect the quality of life and aerobic capacity of patients completely. Researchers believe that physical exercise may mitigate the pain resulting from the disease through some mechanisms (10). Nevertheless, there are correctible factors such as diet and physical activity which support the preventive and therapeutic methods against this disease. Physical activity can have different effects on this disease depending on energy reserves, exercise intensity and periodicity, along with oxidative stress resulting from exercise (11). Studies have shown that use of exercise interventions can be effective for patients with endometriosis (2). Nevertheless, what type of physical activity and through which molecular and cellular mechanisms it can have the best impact are still poorly understood. Research has shown that low-intensity aerobic exercise can lead to decreased expression of inflammatory cytokines, oxidative stress, and systemic inflammation in the uterus through establishing protective mechanisms, thereby inducing immune responses (12). The effect of physical activity on rest levels of IL-1β and COX-2 has not been specified completely. Research indicates that exercise can considerably inhibit COX-2 activity, thus leading to suppression of proinflammatory cytokines and alteration in the oxidation status (13).

Although no change has been observed in the COX-2 protein content according to some studies (14), the results suggest that exercise may, as least to some extent, mitigate the inflammation by reducing its formation. Further, IL-1β variations along exercise can occur in a non-inflammation dependent way. It was also observed that exercise may modulate inflammation formation or activation (15). Nevertheless, it should be noted that no study has explored the effect of physical activity on these two proteins in endometriosis, and the mechanism of their changes is poorly understood. From among aerobic exercises, swimming aerobic exercise with low intensity is among the exercises that can be used under various physiological conditions safely, and due to weight tolerance in water in relation to nonwatery sports, it is used in most physiological, biochemical, and molecular reaction studies. Mild to moderate physical activity can improve metabolic activity through increasing blood circulation, but severe activity, due to blood circulation displacement towards the muscles causes its reduction (10). Meanwhile, research has shown that a diet ricj in fruits and vegetables as well as cereal grains can be effective in prevention from growth of endometriosis metastasis (2). For example, Vitamin B6 is a central molecule in living creature cells. It is an important factor for a wide range of biochemical reactions, which regulates the essential cell metabolism (16). Vitamin B6 has been known as a potent anti-inflammatory, anti-mutagenic, and neuro-enhancer agent; through activating suppressor genes and deactivating angiogenesis as well as activating anti-inflammatory gene in induction of apoptosis causes inhibition of progression of cancerous tumors as well as many pathological changes that occur in response to penetration of inflammatory cells (17).

The group B family leads to pain alleviation as well as improvement in the endometriotic lesion and reduction of this disease symptoms (18). Nevertheless, no research has been performed so far with a focus on the influence of vitamin B6 on endometriosis. Endometriosis is a multifactorial disease with a complex pathophysiology, and most of its details have still remained unclear. Thus, considering the limited studies as well as the discrepancies and ambiguities regarding the effect of dietary and exercise interventions, regarding the preference of each of the mentioned methods and lack of similar studies, the present study was performed to examine the effect of physical activity and vitamin B on the expression of COX2 and IL-1B genes on the endometriotic tissue of endometriosis model rats.

2. Materials and Methods

In this experimental research, 25 adult 6-8-weak-old Wistar rats with the mean weight of 202.85±15.65 g were purchased from Pasteur Institute and transferred to the research center. The animals were kept according to the guidelines of Health International Institute, and the protocols of this study were done observing the principles of Declaration of Helsinki as well as medical ethics considerations. Pellet food and water were provided to the animals under treatment ad libitum. The food consumed by the animals was 10 g per each 100 g of the body weight based on weekly weighing. The protocol of this research was performed according to the international laws of handling laboratory animals. The rats were randomly assigned into five groups (5 rats in each group), including control-healthy group, endometriosis, endometriosis + exercise, endometriosis + B6, endometriosis + B6 + exercise. In order to induce the endometriosis model, first adult rats were anesthetized using ketamine and xylazine. Next, the abdominal region on the right side was cleansed using Betadine. Thereafter, an incision was made in the skin of the flank region in the pelvic part using a Bistoury blade. Once the abdominal muscle and peritoneum were opened, first the ovarian tissue alongside part of the uterine tube tissue were withdrawn. They were then placed inside a sterilized container with 1 cc PBS. Thereafter, each tissue was cut into a 1*1 mm piece. The tissue pieces, which were four for each rat, were grafted to the right pelvic muscular wall region, abdominal peritoneum, abdominal wall frontal region, and the visceral tissue around the ovaries. Next, the operated region was sutured, and the rats were transferred to the relevant cage (19).

Vitamin B6 was administered two weeks after induction of endometriosis on a daily basis and as gavage by 60 mg/kg of body weight in the rats of the endometriosis + B6 and endometriosis + B6 + exercise groups (11). The rats of endometriosis + exercise ad endometriosis + exercise + B6 groups, before initiating the main protocol, were placed inside water pool for 1 week (5 days) each time for 20 min, to get familiar with water and lower their swimming stress and get adapted to the exercise conditions. Then, they began swimming five days a week until the end of the research period inside a water tank with 50 * 50 * 100 cm dimensions over 8 weeks. The duration of exercise in water was 30 min daily until end of the exercise period (20). Once the period finished, in order to eliminate the acute exercise effect, samples were taken from the3 animals 48 h after the last swimming exercise program and consumption of Vit B6. For this purpose, first the animals were anesthetized through intraperitoneal injection of ketamine (20-30 mg/kg) and xylazine (20-30 mg/kg); after dissecting the abdominal cavity, the ovarian tissue was carefully withdrawn and frozen at -80˚C for investigating the expression of COX-2 and IL-1β genes and transferred to the laboratory. In order to examine the expression of COX-2 and IL-1B genes, in each group, the tissues were inspected using Real-time PCR. Next, cDNA was replicated via PCR technique, whereby qRT PCR was employed to confirm the expression of the studied genes quantitatively. After laboratory analysis of the tissue samples, descriptive statistical indices including mean and standard deviation for quantitative description of data along with inferential statistics. First, to determine the normality of data distribution, Shapiro-Wilk test was used, while for determining the variance homogeneity, Leven's test was applied.

Next, since the data distribution was normal, parametric tests including one-way analysis of variance and post hoc Tukey test were used at significance level of p-value≤0.05 to explore the changes in the expression of COX2 and IL-1B genes. For all statistical analyses, SPSS 23 was used, while for drawing the diagrams, Excel software was employed.

3. Results

The results of ANOVA test showed that there was a significant difference between the groups regarding levels of expression of IL-1β and COX genes (p-value≤0.0001, Table 2). The results of post hoc test also showed that the extent of expression of COX and IL-1β genes in the endometriosis group had a significant elevation compared to the healthy-control group (p-value≤0.0001).

All three groups of endometriosis + exercise, endometriosis + B6, and endometriosis + B6 + exercise showed a significant reduction in the extent of expression of COX gene, compared to the endometriosis group (p-value≤0.0001). Two groups of endometriosis + exercise and endometriosis + exercise + B6 revealed a significant decline in the extent of expression of IL-1β gene, as compared to the endometriosis group (p-value≤0.0001). The endometriosis + exercise + B6 group indicated a significant reduction in the extent of expression of COX (p-value=0.034) and IL-1β (p-value=0.035) genes, compared to endometriosis + B6 group. Further, the endometriosis + exercise group showed a significant reduction in the extent of expression of IL-1β gene compared to the endometriosis + B6 group (p-value=0.004) (Table 1).

Table 1: Central indices and distribution of Cox and IL-1β gene expression levels in different research groups

Statistic/Group	Control-healthy	endometriosis	endometriosis+B6	endometriosis+exercise	endometriosis+exercise+B6
Mean ± SD (Cox)	±0.028 0.062	0.749±0.045 ❋	#$0.363±0.091	$0.208±0.065	$0.186±0.033
Mean ± SD (IL-1β)	±0.045 0.219	0.871±0.076 ❋	#0.601±0.356	&$0.237±0.087	$0.293±0.066

*. Significant changes in relation to the control-healthy group, $: significant changes in relation to the endometriosis group, &: significant changes in relation to the endometriosis + B6 group, #: significant changes in relation to the endometriosis + exercise + B6 group.

Table 2: ANOVA test results for expression of Cox and IL-1β genes between different research groups

variables	Source of changes	Sum of Squares	df	Mean Square	F	p-value
(Cox)	Between Groups	2.552	4	.365	54.029	≤0.0001
	Within Groups	.216	20	.007		
	Total	2.768	24			
(IL-1β)	Between Groups	3.007	4	.430	20.438	≤0.0001
	Within Groups	.672	20	.021		
	Total	3.679	24			

P<0.05 significant difference

4. Discussion

The present research explored the effect of a period of regular swimming activity along with Vit B6 consumption on the expression of COX2 and IL-1B genes on the endometriotic tissue of endometriosis model rats. The results revealed that with induction of the endometriosis model, the expression of COX-2 and IL-1B genes in rats increased significantly compared to the control group. On the other hand, with implementation of the exercise and B6 treatment, the expression of the mentioned genes in rats showed a significant reduction compared to the endometriosis group, with this decline being greater in the exercise + B6 combined group. It has been reported that the endometrium completes a three-stage method (binding – aggression – angiogenesis), and eventually converts to endometriosis (21).

Recent studies have shown that immunological, inflammatory, angiogenetic, and environmental factors all can be heavily implicated in the pathogenesis of endometriosis. In addition, the peritoneum in patients with endometriosis is immunologically dynamic, linking the reproductive and immune system with each other. There is evidence suggesting that the peritoneal fluid around the endometriosis implant has pro-inflammatory tissue features, associated with altered immune response and cytokine production. It has been suggested that these cytokines with autocrine and paracrine effects may impair the intercellular interactions in immune cells, and include proliferative factors for implantation and development of extrauterine tissue (22). IL-1B is the most important anti-inflammatory cytokine, which reinforces the inflammatory state in endometriosis, causing secretion of other cytokines and growth factors, eventually supporting implantation and development of extrauterine lesion (21).

Endometriosis is a gynecological inflammation-dependent disorder. Overexpression of COX2 plays a key role in the development and progression of endometriosis. Nevertheless, the mechanism of endometriosis is unclear (21). In this study, similar to some studies, overexpression of COX-2 in extrauterine endometrium of patients with endometriosis was observed against the normal endometrium of the control group (22). Studies show that COX-2 plays a key role in the pathology of endometrium, especially in endometriosis. Expression of COX-2 is higher in local lesions of endometriosis than in endometrium, indicating increased synthesis of COX-2 enzyme and hence production of PGE2 in the peritoneal fluid. It has been reported that COX-2 is specifically regulated by IL-1b in endometrioses compared with normal endometrium stroma. Thus, these data support overexpression of COX-2 in extrauterine lesion and hypersensitivity of COX-2 expression resulting from IL-1b in endometrioses, which may aid the pathophysiology of endometriosis (21). Nevertheless, factors such as nutrition and physical activity help in prevention and treatment through regulating and modulating this disease (2). Research has shown that physical activity can reduce the risk of developing endometriosis in women. Some possible mechanisms introduced in this regard include decrease of oxidative stress, strengthening the immune system, and modification of hormonal factors (23). In addition, regular physical activity is associated with cumulative effect of menstruation, ovary stimulation, and estrogen functioning (11). Research indicates that exercise considerably inhibits COX-2 activity, and leads to suppression of proinflammatory cytokines and changes in oxidation status.

These results suggest that there is a molecular relationship between the central nervous system and the body's immune system (13). IL-1β is one of the most potent and important inflammatory mediators in acute phse response and in pathophysiology of chronic diseases (24). Data show that reduction of inflammatory cytokines following exercise intervention may occur through reducing the IL-1β activation (15). Recently, a review study on the relationship between vitamins and endometriosis disease has shown that there is a direct relationship between vitamin groups and improvement of endometriosis, whereby group B vitamin results in pain alleviation and improvement of endometriotic lesion as well as reduction of symptoms of this disease. Vitamin B6 has been known as a potent anti-inflammatory, anti-mutagenic, and neuro-enhancing factor (18). Group B vitamin has a key role in its prevention and treatment through modulating cellular signaling pathways. Since B6 vitamin has antioxidant properties, following exercise, it encourages rapid regeneration of exercise-induced damaged cells; it causes the body to be able to absorb fat-soluble nutrients, and lead to vasodilation, thereby facilitating blood flow (2). Vitamin B6 is considered essential for normal metabolism and immune response especially anti-inflammatory immune response. A study reported that Vitamin B6 inhibits expression of lipopolysaccharide (LPS) resulting from synthesis of nitric oxide synthase (iNOS) and COX-2 in mRNA as well as protein level through suppressing NF-kB activation in macrophages (25). Recent studies have shown that in groups undergoing treatment with B6 vitamin in comparison to the control group, expression of IL-1β, TNF-α, and IL-6 decreased. The results suggest the protective role of Vit B6 against hyperinflammation (26).

Conclusion

Overall, the results suggest that alteration of key molecules or signaling pathways as well as gene expression involved in endometriosis process can improve the level of this disease. Doing regular aerobic exercise as well as concurrent consumption of Vit B6 can be effective in mitigating this disease as well as its improvement.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval The Ethics Committee of Islamic Azad University-Sari Branch approved the study (IR.IAU.SARI.REC.1399.120).

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: F.R., P.F., H.A.; Methodology: F.R., P.F., H.A.; Software: F.R., H.A.; Validation: F.R., P.F., H.A.; Formal analysis: F.R., P.F., H.A.; Investigation: F.R., P.F., H.A.; Resources: F.R., P.F.; Data curation: F.R., P.F., H.A.; Writing - original draft: F.R., P.F., H.A.; Writing - review & editing: P.F., H.A.; Visualization: F.R., P.F., H.A.; Supervision: F.R., H.A.; Project administration: F.R., P.F., H.A.; Funding acquisition: F.R., P.F., H.A.

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