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ORIGINAL ARTICLE

Effect of 12-week Aerobic Exercise on the Tumor Size and Expression of HIF-1α, BCL-2, Mir-15a, and VEGF Genes in BALB/C Female Mice with Breast Cancer

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KEYWORDS

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ABSTRACT: Angiogenesis and mortality are associated with breast cancer, one of the most common tumors in women. Tumor angiogenesis is affected by exercise. This study examined the effects of 12 weeks of exercise on HIF-1α, mir-15a, BCL-2, and VEGF gene expression in BALB/c female mice. Forty BALB/c (two week's age) female mice with a mean weight of 17±0.21g were separated into control and treadmill aerobic training groups. Mice developed malignant after receiving 200µL of MC_4-L_2 cells subcutaneously. Running at 15-20m min^{-1} for 10 weeks was the aerobic activity. Afterward, mice were killed and tumor tissue RNA was extracted. HIF-1α, mir-15a, and VEGF gene expression in BALB/c female mice were measured using quantitative real-time PCR (RT-qPCR). After 12 weeks of exercise, miR-15a expression rose 2.6 fold, whereas HIF-1α, Bcl-2, and VEGF gene expression dropped 3.1, 2.6, and 2.4 fold, respectively ($p<0.05$). Exercise can activate pathways that slow breast cancer progression. More research is needed to confirm these findings and other molecular pathways.

INTRODUCTION

One in six people lose their lives to cancer in 2020, and while the disease is responsible for roughly 10 million fatalities overall that year, 2.26 million of them will be due to breast cancer. Deaths attributable to breast cancer are expected in 2020 (685 000 deaths) [1]. The cancer rate has increased during recent decades and breast cancer includes one of the most devastating, most prevalent and fatal cancer types among women patients [2-4].

Breast, lung, colon, rectal, and prostate cancers account for over half of all cancer diagnoses [5]. Tobacco use, excess weight, alcohol use, insufficient diet of fruits and vegetables, and insufficient exercise all contribute to around a third of all cancer-related fatalities [6]. Early detection and good treatment of many malignancies make a cure possible. Cancer is an umbrella word for a wide variety of illnesses with potentially devastating effects on every organ system. Malignant tumors and neoplasms are other names for cancerous growths [7]. Cancer is characterized by the fast generation of aberrant cells that expand beyond their normal borders, invading neighboring tissues and eventually spreading to other organs; this process is known as metastasis. The leading cause of

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cancer-related mortality is metastases that spread throughout the body [8].

Among the major risk factors for the initiation and development of breast cancer, change in lifestyle such as being overweight, low motility rate and obesity can be mentioned [8,9]. Metastasis is the main cause of death from cancers through using various mechanisms [9, 10]. The overweight is associated with the increase of estrogen release from the fatty tissues which can hinder apoptosis or programmed cell death in cells and enhance the cell proliferation. Thereby, the mitigation of the apoptosis in cells is associated with the increase of breast cancer which can be exerted by various mechanisms. The disturbance in the pro-apoptotic (BAX) or anti-apoptotic (Bcl-2 and Bcl-XL) proteins function, low caspase enzyme function or death receptor also causes cell immortality [1].

Various studies have been performed on the effect of exercise on tumor growth via alterations in the gene expression profile. Moreover, the preventive effects of exercise on cancers have been demonstrated. The angiogenesis of tumor tissue and blood supply is a paramount event for the persistence and growth of the tumor tissue. Considering this, vascular endothelial growth factor (VEGF) plays a crucial role as the mitogen agent in the angiogenesis of breast cancer and also causes multiplication, metastasis, and the detachment of the endothelial cells matrix, formation of vascular networks and nitric oxide production and distribution in the endothelial cells [11, 12]. Additionally, VEGF has an anti-apoptotic effect on the endothelial cells, enhanced the penetrability and vasodilation. Several microRNAs have also participated in tumor growth, such as miRNA206, Let-7 and miR-21.

The response to an external stimulating agent such as exercise may be due to the inhibitory effects of miRNAs on gene expression which necessitates further deep studies. miRNAs as part of cancer in cancer's progress via various mechanisms such as apoptosis, differentiation, cell signaling, survival, ageing and metabolism. miR-15a is example of inhibit tumor growth by the effect on pro-apoptotic and anti-apoptotic genes. Hypoxia-inducible factor 1 (HIF-1α) has a substantial role in the cancer progress and metastasis which limits the oxygen levels and provides the growth of tumor tissue [13-15].

There is ample evidence that regular moderate to vigorous aerobic physical activity is related to a reduced risk for various forms of cancer to suggest a causal relationship[16]. Exercise is associated with positive changes in fitness, body composition, and physical functioning as well as in patient-reported outcomes such as fatigue, sleep quality, or health-related quality of life. Emerging evidence indicates that exercise may also be directly linked to the control of tumor biology through direct effects on tumor-intrinsic factors [16].

Many cancer survivors report persistent fatigue long after their treatment has ended. Previous research by Patel, et al., 2020 [17] has demonstrated that intervention is more effective in combating weariness. Fatigue in people with cancer is called cancer-related weariness (CRF), and it's very different from the fatigue experienced by healthy people. The symptoms of CRF are out of proportion to the amount of effort the patient puts out, and rest and sleep do not always help. Patients with solid tumors who engaged in aerobic exercise for 6 weeks following chemotherapy and/or radiation therapy had a reduction in the kind and severity of tiredness experienced by these individuals. Cancer-related tiredness is reduced, and physical function is enhanced, as measured by a longer 6-minute walk distance (6MWD). After aerobic exercise, these patients also reported a notable rise in their overall quality of life [17, 18]. Aerobic exercise is an effective non-pharmaceutical anticancer intervention [19]. The aim of this study was to evaluate the 12 weeks of exercise and check their effect on the expression of HIF-1α, mir-15a, Bcl-2 and VEGF genes in BALB/c female mice.

MATERIALS AND METHODS

Lab animals

Forty BALB/c, two weeks old, female mice with a mean weight of 17±0.21g were divided into two control and aerobic exercise. The mice were kept in suitable conditions and with normal food and water supply. Using the

subcutaneous injection of MC_4-L_2 cells (200µL) on the back of mice, the mice became cancerous.

Exercise stage

The aerobic exercise included 12 weeks with 10 sessions of running with a speed of 15-20m min^{-1}. After 24 h of the last exercise, the tumor tissue was extracted, weighed, and kept at -70°C. Next, the mice were euthanized and tumor tissue RNA was extracted. RNA extraction from the tumor tissues was performed using the RNeasy Mini Kit (Qiagen, Germany), and total RNA Extraction Kit according to the instructions of the manufacturer.

The expression of genes

Moreover, the Easy cDNA Synthesis Kit, Qiagen cDNA synthesis kit was used for cDNA synthesis as per the protocols of the manufacturer. The expression of HIF-1α, miR-15a, BCL-2 and VEGF genes in BALB/c female mice was assessed using quantitative real-time PCR (RT-qPCR) (Step one plus AB Applied Biosystems). The primer sequences have been shown in Table 1. Normalized, arbitrary values were established for each gene using GAPDH as the reference gene. Data analysis was performed using the $2^{-\Delta\Delta Cq}$ method.

Table 1. The primer sequences and products in this study.

Primer	Sequence: 5ˈ to 3ˈ	Annealing (°C)	Reference
HIF-1α	F: TATGAGCCAGAAGAACTTTTAGGC	60	[20]
	R: CACCTCTTTTGGCAAGCATCCTG		
BCL-2	F: CCCTGGTGGACAACATCG	60	[21]
	R: CAGGAGAAATCAAACAGAGGC		
VEGF	F: TGAACTTTCTGCTCTCTTGGG	55	[22]
	R: GGTTCGCTGGTAGACATCG		
miR-15a	UAGCAGCACAUAAUGGUUUGUG	60	[23]
GAPDH	F: AGGCCGGTGCTGAGTATGTCGTG	56	[24]
	R: TCACAAACATGGGGGCATCGG		

Notes: HIF-1α= *Hypoxia-inducible factor 1*-alpha; BCL-2= B-cell lymphoma 2; VEFG=Vascular endothelial growth factor; miR-15a= microRNA-15a; GAPDH= Glyceraldehyde-3-Phosphate Dehydrogenase; F= forward; R= reverse.

RESULTS

After 12 weeks of exercise, 24 of the last training exercises the tumor tissue was separated, weighed and then kept at -70°C. The size increased gradually in the control group the exercise group (Figure 1).

The expression of miR-15a was increased 2.6 fold, while the expression of HIF-1α, Bcl-2 and VEGF genes was decreased 3.1, 2.6 and 2.4 fold, respectively in the exercise group compared to those in the control population (Figure 2).

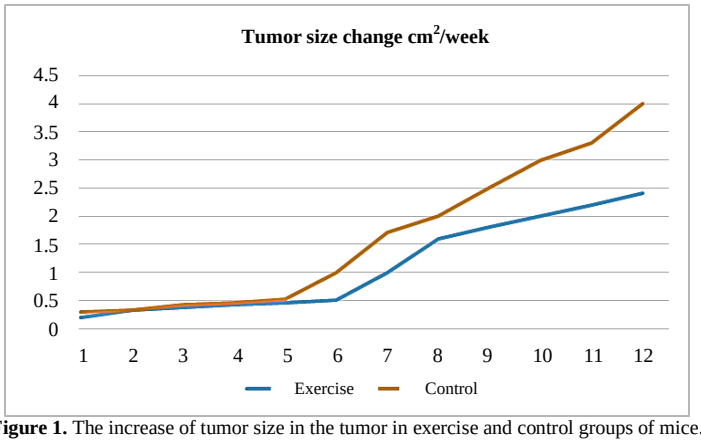

Figure 1. The increase of tumor size in the tumor in exercise and control groups of mice.

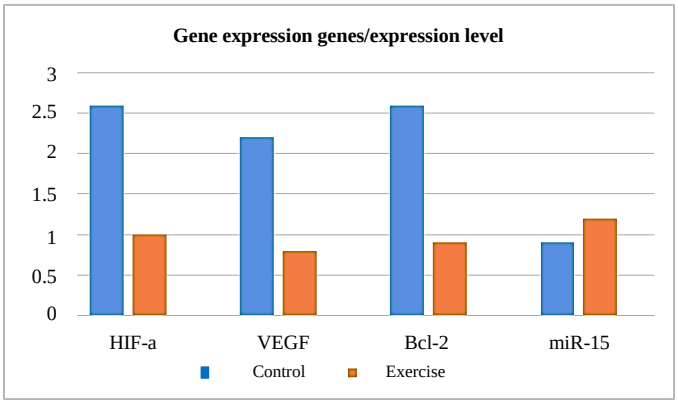

Figure 2. The expression of miR-15a, HIF-1α, Bcl-2 and VEGF genes in the exercise and control groups.
Notes: HIF-1α= *Hypoxia-inducible factor 1*-alpha; BCL-2= B-cell lymphoma 2; VEFG=Vascular endothelial growth factor; miR-15a= microRNA-15a.

DISCUSSION

According to the data obtained from tumor volume, the present study showed that aerobic exercise can reduce the progression of breast cancer and its size. Due to the increased expression of miR-15a gene and decreased expression of HIF-α, VEGF and Bcl-2, a link between these several regulatory factors recognized as a new mechanism for creating positive effects of aerobic exercise on breast cancer is considerable [25-27]. In recent years, new areas of physical activity have emerged in developed countries that approach a therapeutic approach; For example, research has shown that regular exercise can reduce tumor volume, which was also confirmed in the present study the tumor growth was significantly lower in the exercise group. Consistent with the present study, the results of another study also showed that aerobic exercise by altering the expression of miR-15a and Bcl-2 genes as a treatment can stimulate tumor growth in groups that are active after cancer in the exercise group had been reduced compared to other groups, by up to two times, however, in this study, the cause was not mentioned. It was also shown that intense activity affected the tumor microenvironment and growth, leading to growth retardation [28-30]. In addition, a previous study by [31, 32] Exercise has been found to decrease tumor volume by reducing angiogenesis, VEGF expression, erythrocyte counts, and lactate in the tumor microenvironment while increasing oxygen and nitric oxide levels. Studies on mice with breast cancer have also shown that exercise significantly reduces the number and volume of tumors. In the present study, the decrease in tumor growth ratio was attributed to the suppression of Bcl-2, VEGF, and HIF-α gene expression, as well as the increase in miR-15a expression. This study indicates that miR-15a expression was significantly higher in the exercise group than in the control group post-translation, and it plays an important role in cancer due to its location and expression profile [33, 34]. Genetic changes such as the absence of heterozygosity and homozygosity in more than half of cases of various cancers involving abnormal chromosomal frequencies have been identified. More than half of all miRNAs are in cancer-dependent genomic regions caused by mutations or meaningless expression in miRNAs. Abnormal expression of miRNAs alters the expression of genes encoding proteins that are involved in tumorigenesis and tumor suppression and cause various cancers [28, 29, 33, 35, 36]. The results of studies performed on solid tumors have demonstrated that miR-15a has been eliminated or reduced in tumor cells. Recent studies exhibited that miR-15a is used in many cancers, such as leukemia, prostate cancer, osteosarcoma, pituitary adenomas and breast cancer. Keratocystic androgenic tumors and has a regulatory role [37]. These data support the hypothesis that miR-15a is altered in such as osteosarcoma, and androgenic keratocystic tumors. The effect of 12 weeks of aerobic exercise on the expression of Bcl-2 and miR-15a genes was observable. It acts as a tumor suppressor, and this reduction is involved in tumorigenesis.

miR-15a participates in a variety of gene targets involved in cell proliferation and survival, including CCND1, WNT3A, MCL1 and Bcl-2 genes [38, 39]. Also, miR-15a binds to its target site in the untranslated region of '3' UTR'-3 (CCNE1), which decreases CCNE1 mRNA and protein levels in estrogen receptor-positive breast cancer cells, resulting in inhibition of cell growth, suppression of migration, and cell cycle arrest [37]. In addition, miRNA-15 overexpression reduces Bcl-2 expression and, consequently, apoptosis in MCF7 breast cancer cells [40, 41]. Bioinformatics tools have shown that the two products are homologous to each other. These findings indicate that Bcl-2 is one of the post-translational targets of miR-15a. Bcl-2 is also a central oncogene in the genetic program of eukaryotic cells surviving by inhibiting cell death [42]. The overexpression of Bcl-2 protein has been observed in various cancers. Also, miR-15a with a post-translational effect on Bcl-2 decreases its regulation [43]. The decrease in Bcl-2 protein observed in the present study can be rooted in miR-15a, which is increased due to aerobic exercise. The result of these changes is certainly a reduction or a delay in tumor growth. In light of the above, it is clear that exercise has beneficial effects on the treatment of breast cancer or secondary diseases associated with cancer, but so far little research has been done on molecular and cellular mechanisms [42]. The beneficial effects of exercise on breast cancer tumor tissue have been investigated and the message pathways and effective mechanisms of exercise activity in curing cancer are not known. The results of this study have revealed that aerobic activity can affect the state of apoptosis within cancer cells Specifically, aerobic exercise can increase miR-15a expression and decrease Bcl-2 gene expression and protein levels in mice with hormone-dependent breast cancer. These findings suggest a new mechanism for expressing the positive effects of exercise on breast cancer treatment, which should be considered.

CONCLUSIONS

The findings of this study showed that regular aerobic exercise can be used as a complementary treatment alongside other breast cancer treatment. However, in order to better understand the molecular and cellular mechanisms involved in the beneficial effects of regular exercise on tumor tissue in breast cancer, further studies are needed. Exercise has the potential to activate mechanisms that reduce breast cancer, but more detailed studies are required to fully verify these results.

ETHICAL CONSIDERATION

Permission to conduct this study was issued by the Fasa University of Medical Sciences, the University of Fallujah, and the University of Misan.

Conflict of interest

None

Abbreviations

HIF-1α= *Hypoxia-inducible factor 1*-alpha; BCL-2= B-cell lymphoma 2; VEFG=Vascular endothelial growth factor; miR-15a= microRNA-15a; GAPDH= Glyceraldehyde-3-Phosphate Dehydrogenase; F= forward; R= reverse.

Authors' Contribution

All Authors Contributed equally.

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