

Comparison of protective effect of two types of aerobic and intermittent training on breast cancer by TGF β protein and Smad-3 gene and MMP2 in female mice

Vahid Moghaddam¹ Maghsoud Peeri¹ Mohammad Ali Azarbayjani¹ Hasan Matin Homaei¹

Department of Exercise Physiology¹, Central Tehran Branch, Islamic Azad University, Tehran, Iran.

(Received 8 Feb, 2017)

Accepted 15 May, 2017)

Original Article

Abstract

Introduction: The aim of this study was to compare the protective effects of two types of aerobic and intermittent training on breast cancer as a result of TGF β protein, Smad-3, and MMP2 gene in female mice.

Methods: In the experimental study, a total of 24 female BALB/c mice after Tumorigenesis by MC4-L2 cell line in the three groups; Control, Aerobic, and Intermittent training groups, and under standard conditions were studied. Aerobic continuous training group performed 60 minutes of running on a treadmill with intensity of 60-65% VO₂max, 5 days a week and high-intensity intermittent program included six bouts alternates (3 minutes and 20 seconds with intensity of 85-90% VO₂max and a minute of recovery with intense 30-35% VO₂max between each alternate) for a period of 10 weeks. Data using one-way ANOVA and Tukey's test at a significance level of P<0.05 were analyzed.

Results: High-intensity intermittent training significantly increased expression of Smad-3 (P<0.03), and protein TGF- β (P<0.000) and decreased the expression of MMP-2 (P<0.000) in tumor tissue than in the control group. Aerobic training also significantly increased the expression of Smad-3 (P<0.03) and protein TGF- β (P<0.000) and decreased the expression of MMP-2 (P<0.04) in tumor tissue than in the control group. No significant differences in gene expression Smad-3 and MMP-2 between training groups was observed (P<0.05), However, there was significant differences in the TGF- β protein between High-intensity intermittent training and aerobic training groups (P<0.05).

Conclusion: It seems that high-intensity intermittent and aerobic continuous training can possibly be effective interventions to reduce breast cancer progression and there is not much difference between the effects of two types of training.

Key words: Breast Cancer, Training, TGF β , Smad, MMP2

Citation: Moghaddam V, Peeri M, Azarbayjani MA, Matin Homaei H. Comparison of the protective effect of two types of aerobic and intermittent training on breast cancer by TGF β protein and Smad-3 gene and MMP2 in female mice. Hormozgan Medical Journal 2017;21(1):47-57.

Introduction:

Cancer is one of the most common diseases in the civilized world and the number of patients is on

the rise every day (1). Despite significant advances in medical science, cancer continues to be one of the fatal diseases of this century, and the second leading cause of death after cardiovascular diseases

(2). The breast cancer is the most common cancer among women in developed and developing countries (2).

We can point to some biomarkers in cancer development, simple tests for early diagnosis and prognosis of disease, and its severity and response to treatment can play a different and essential role. Among the factors affecting are MMPs which are measured in tumor tissue types (3). The role of proteases and especially Matrix metalloproteinase (MMPs) in metastasis and invasion of tumor cells and completely proven and known that these enzymes help this process by facilitating the breaking of the connection connective tissue, and digesting and extracellular matrix components (4). Among this group, the Matrix metalloproteinase -2 (MMP2), in many normal and deformed cells and some malignant cells is generated which can effect on collagen membrane based on cellular and denatured collagen and gelatin, elastin, proteoglycan, laminin and etc (5). On the other, Transforming Growth Factor β (TGF- β) is one of the most powerful natural inhibitors of cell proliferation is vital to its oncogenic activity. Autocrine and paracrine effects of TGF-beta in the tumor cells and the tumor micro-environment is showing both positive and negative effects on the development of the cancer. Accordingly, TGF- β signaling pathway as a tumor suppressor and promoter of tumor progression and invasion route was considered. In normal breast epithelial cells and in the early stages of cancer progression, TGF- β with its suppressive effect on tumor growth and through the inhibition of cell proliferation acts as a growth inhibitor. But with a progress of cancer, the function of oncogenic and tumor-inducing effect is generated and therefore it causes an increase in the potential of invasion and metastasis.

The resistance of cancer cells to the suppressive effects of TGF- β signaling pathway occurs through different mechanisms (6,7). As described, TGF- β is a potential induction expression and is a very important factor in cancer development. This signaling pathway induces the outcomes by Smad-dependent and non-dependent Smad pathways. Receptors TGF- β (TGF- β R1, R2) are the dual acting kinase, which has the activity of tyrosine kinase and also show the activity of threonine/serine kinases. After closing the complex heterodimeric

receptors TGF- β R1, R2 is an informative signal cascade that begins with phosphorylation of R2 and causes Auto phosphorylation serine TGF- β R1 in which, a number of phosphorylation of specific proteins (especially family of transcription factors Smad) become active. Smads bind to a type of Co-Smad Called Smad4. Complexes 2/3 Smad along with minor transcription factors can activate genes involved in differentiation (8,9).

Throughout the years, researchers sought to find the best solution for the treatment of this disease. In this regard, the impact of nutritional factors (10), pharmaceutical (11) and sport (12-15) on breast cancer has been studied. According to the studies, the field of physical activity is shown as a safe intervention to improve the quality of life and treatment for breast cancer patients (16), but it is still the controversial aspect of treatment. That's why identifying the impact of physical activity on cancer pathways could be an important step in proving its role in the control and treatment of breast cancer. In this regard, some studies have shown the positive effects of exercise on breast cancer (12-15). Donnelly et al (2014) reported the reduction in MMP following the eight-week physical activity (17). However, the results of Leith et al (2013) studies showed the increase in MMP2 after 12 weeks of resistance training in mice (18). Little research has been done on the effect of physical activity on MMP2. The impact of interval and continuous physical activity on the MMP2 in cancer patients is limited as well. In this regard, according to TGF β protein's role in making adaptations to exercise, the impact of different training methods is taken into consideration. Research showed that TGF- β 1 levels in gastrocnemius muscle tissue after Intensity interval training in male Wistar rats increases (18). In extreme sports activities, exhaustive serum levels of TGF-B significantly increase as well (19). However, 8 weeks of resistance training showed no significant changes in serum levels of TGF- β (20). It is actually yet to be determined whether aerobic continuous and intensive interval training on subjects with breast cancer affects the amount of TGF β protein. In general, investigation in respect of Smad and its relationship to physical activity in disease conditions such as cancer requires further

investigation. A study has shown that resistance exercise increases levels of mRNA Smad (21).

An active lifestyle can reduce the risk of developing breast cancer. Studies show that active women have less chance of developing breast cancer than non-active ones (22). Although several mechanisms to explain this decrease, but there is little evidence to prove each (23). The interaction of changes in gene expression and oncogenic agents with anti-tumor activity in breast cancer are not well defined as well. Most research is done on exercise and cancer effects of exercise on quality of life, muscle strength and endurance and performance indicators in cancer patients, and very few studies on the molecular mechanisms and the effects of exercise on cellular signaling pathways that influence tumor growth is done. As of now, there were no investigations carried on the effects of exercise, particularly aerobic continuous and intensive interval training on the Smad protein and gene expression TGF β and mmp2 in patients with breast cancer. According to mentioned cases, the research intends to evaluate the effect of 10 weeks of aerobic continuous training and high-intensity intermittent TGF β protein and describe gene expression Smad and mmp2 in mice with breast cancer. And it also examines the comparison of the protective effects of exercise on these two factors in samples of breast cancer patients.

Methods:

Among population of female BALB/c mice with breast cancer, 24 rats (6-8-weeks-old, initially weighing 18-20g) have been chosen as statistic samples. The statistical sampling of this research was targeted sampling method, according to weight and age. The rats then were randomly divided into three groups, with eight rats in each group (Table 1): The control group did not participate in any exercise program, but to create the same environmental conditions were put on non-motion treadmill five times a week for 10 to 15 minutes per session for adaptation. Sports groups' aerobic continuous and intensive interval did the training for 10 weeks. Samples tested during the course of a week to get familiar laboratory environment and the treadmill, and as well as the implementation of the Protocol in the form of groups of five rats in a cage

in transparent polycarbonate, in an environment with a temperature of 20-24°C, humidity 45-55% brightness and darkness cycle of 12:12 hours were kept. To absorb urine and feces samples and make them comfortable and clippings, wood chips were sterile. Cleaning cages and wood chips were changed daily.

Table 1. The number of subjects and age groups

Groups	Control	Aerobic continuous training	Intense interval training
Number	8	8	8
Ages (weeks)	6-8	6-8	6-8
Weighing	18-20	18-20	18-20

Breast duct carcinoma cell (MC4-L2) were cultured in T75 flasks in medium DMEM/F-12 with 15 mm HEPES buffer, glutamine, penicillin, Streptomycin and FBS% 10. After filling 90% of the flask by cell supernatants were harvested and then Washing with PBS, then with the enzyme trypsin 0.25% of the floor plate cells were isolated, and after neutralizing the enzyme with medium containing 10% FBS All contents the flask was poured into Falcon tubes and centrifuged at a speed of 1200 for 3-5 minutes, in next step, supernatant was removed and the cell plate was dissolved in 10% FBS medium. Then, to determine the living cell count remained contained, and counted by using of Trypan blue and hemocytometer slide respectively (24).

BALB/c female mice which were supplied by the Pasteur Institute of Iran. After culturing the cell line MC4-L2 and the preparation of a cell suspension, about one million cells were suspended in PBS were injected subcutaneously into the right side of the mice, and then breast tumors appear after 8 to 12 days (24).

Measurement of tumor volume

After the injection of cancer cells and tumor genesis, tumor volume was measured every week. For the measurement of tumor volume from used of the Jones et al. formula $[V = 1/2(L \times W)]$. The tumor volume was measured in two dimensions. After the tumor was considered as the largest tumor and the other (at 90 degrees) as the matter was considered (25).

Aerobic continuous training protocol

Each session includes 70 minutes of continuous endurance exercise program running on a treadmill. The steps include heating stage, including 5minute warm-up intensity, was 30-40% VO₂max. The main phase of training consists of 60 minutes of running at 60-65% VO₂max intensity. The 5minute cool-down with 30-40% VO₂max was considered.

Intense interval training protocol

Each session of interval protocol consists of 35 minutes running on a treadmill. The steps include heating stage, which was 5minute warm-up with intensity at 30-40% VO₂max. The main stage exercise included six alternates (three minutes and 20 seconds with an intensity of 80-95% VO₂max, and one-minute recovery between each period of 30- 35% VO₂max). The 5minute cool-down with 30-40% of VO₂max was also considered.

Stages of tissue sampling and measurement of protein and gene expression

Mice anesthetized with intraperitoneal injection of ketamine [90 mg/kg] and xylazine [10 mg/kg] 24 hours after the last training session. And then they were sacrificed, blood samples, collected directly from the heart mice, and serum isolation by centrifugation at C 3000g, 10, 4 min was performed. The tumor tissue was extracted and immediately frozen at -80 nitrogen and stored for later analysis.

Gene expression measurement of Smad and mmp2 by REAL TIME PCR method.

To get even and uniform solution, 0.7 g of agarose powder in 70 ml of buffer TAE (1X) boiled for 2-3 minutes in the microwave oven. Then 3.5 ml DNA safe stain is added to the gel before hardening. And gel contents poured in the specially molded gel (Tray) container, and special comb put in it and we wait to become the fully-formed gel.

Total RNA extraction process were as follows:

1) Homogeneous tissue, 2) the isolation of RNA, 3) washing the RNA, 4) suspension of this, 5) and reading the OD with Nanodrop. RNA quality control was performed using an agarose gel electrophoresis. After preparing the gel, it was placed in the tank for electrophoresis. And it is

poured in the tank, buffer TAE (1x), until it covers even or a bit higher than the gel. The comb is slowly withdrawn and the samples and the molecular weight markers in special loading place for sampling were placed. Electrophoresis was performed at a voltage of 120 for 60-90 minutes and maximum 18s to 28s bands under fluorescent light using gel doc for observation (26).

c DNA synthesis for gene Smad and mmp2

At this stage, the reaction product, DNase Treatment which contains 11 microliters of RNA, and 1 microliter of random primers was added for 10 minutes at 65°C. After the incubation time the reaction product was put on ice, and for each micro-tube, four microliters of 5X Reaction Buffer, 0.5 microliters RiboLock RNase Inhibitor (20 u/μl), 2 microliters dNTP Mix and 0/5 microliters (200 u/ul RevertAid) M-Mul Reverse Transcriptase is added. And then put in Corbett thermocycler. Temperature and reaction time based on the kit were: 25°C for 10 minutes, 50°C for one hour, 85°C for five minutes and finally the reaction product for subsequent reactions - 80°C maintained (26).

First synthesized cDNA for IGF-1, IGF-1R with 40 microliters of RNase & DNase -free water was diluted. 1.5 microliters of each of the dilutions with 7.5 microliters Master Mix produced by ampliqon (Denmark) and one microliter of Forward primers and one microliter of backward primers in four microliters nucleate-free water to reach the final volume of 15 microliters solution were mixed well. All reactions to form pairs (duplicated) were carried out. Finally, micro-tubes were put in their own special places in the device and proliferation reactions in 40 cycles according to the manufacturer's instructions kit was performed as follows: at 95°C for 15 seconds and at 60°C for 60 seconds. It should be noted that the initial denaturation temperature and time were 95°C and 10 minutes respectively. Finally, standard graph and proliferation primers in the system were analyzed and drawn by the software. To be certain of the outcome, reaction product melting temperature curve was also drawn. Proliferation response of every specified gene in this study, as described above is done, with this difference that the proliferation response was used for only to a

dilution of the sample (optimal dilution) model. To study the gene expression changes qRT-PCR response was performed in the same order. GAPDH was used for internal control gene and for quality control of reaction product of GAPDH related to sample on the gel 2% of it was transferred to investigate presence or absence of it (26).

Quantification of target gene expression levels

To quantify the levels of gene expression the $2^{-\Delta\Delta CT}$ method was used. In this method, necessary values were obtained through the process and were placed in the method and And fold change values were calculated.

$$\Delta Ct = Ct \text{ (a target gene)} - Ct \text{ (a reference gene)}$$

$$\Delta\Delta Ct = \Delta Ct \text{ (an experimental sample)} - \Delta Ct \text{ (a control sample)}$$

$$2^{-\Delta\Delta Ct} = \text{gene expression changes compared to the control group}$$

Extraction of total cellular protein by Western Blot

In this study, to evaluate the protein TGF- β and β -Actin total cellular proteins were extracted first. The approximate value of 106×10^6 cells per flask by centrifugation (2000 rpm for 5 minutes and the temperature of 4 °C) were taken and washed with cold phosphate saline buffer. Then for every cell sediment with respect to deposited cells, a certain amount of lysis buffer cell (containing 1% NP-40, 0.5% SDS, 10 millimolar Tris-HCl (pH: 7.4), 150 millimolar NaCl, 5 millimolar EDTA, 5.0% sodium deoxycholate, 100 millimolar PMSF and protease and phosphatase inhibitors) were added. Samples were placed on ice for 30 minutes at a temperature of four degrees Celsius. The specimens were Vertex every five minutes. After this phase, cell samples were centrifuged for 20 minutes at a temperature of four degrees Celsius at 13000 rpm (27).

To ensure the normal distribution of data Kolmogorov-Smirnov test was used. Then, to determine the significance of differences in the

expression of each training group and the control group as well as to study the differences between the three groups T-independent test was used, in another way ANOVA test for homogeneity of variances and determining the position of significance in post hoc Tukey test was used as well. All statistical operations of research using SPSS version 22 with the significance level of $0.05 > P$ were considered.

Results:

The values in Table 2. The mean and standard deviation of tumor volume are shown in research groups in separate weeks.

In Table 2. The mean and standard deviation of the expression of MMP-2 Smad-3, in the group's research, is stated as well. Independent t-test results showed that aerobic exercise ($P < 0.04$) and high-intensity interval ($P < 0.000$) significantly decreased the mean value of expression of MMP-2 in tumor tissue compared to the control group. The results also showed that aerobic exercise ($P < 0.000$) as well as high-intensity interval training ($P < 0.000$) a significant increase of mean value in TGF- β protein concentration in tumor tissue compared to the control group.

In addition, aerobic training ($P < 0.03$), as well as high-intensity interval training ($P < 0.007$), caused a significant mean value increase in the expression of Smad-3 in tumor tissue compared to the control group. ANOVA test results showed significant differences between gene expression of MMP-2, Smad-3 and TGF- β levels between the experimental and control groups ($P < 0.05$).

Tukey test showed a significant difference between the two groups of high-intensity intermittent training and control groups, as well as continuous endurance and control group in the gene expression of MMP-2 ($P < 0.05$). But among training groups, there are no significant differences in the expression of MMP-2 gene ($P > 0.05$) (Figure 1).

Table 2. Mean tumor volume (tumor volume in mm3) in different

Group Week	Intensity interval training	Aerobic continuous training	Control
1	177.44 ± 49.25	121.04 ± 19.78	108.38 ± 24.39
2	208.43 ± 51.81	174.11 ± 30.34	159.67 ± 26.13
3	279.65 ± 58.55	221.82 ± 32.97	239.37 ± 29.98
4	375.58 ± 74.76	284.64 ± 27.42	379.19 ± 43.22
5	531.53 ± 81.15	377.64 ± 42.89	478.09 ± 35.70
6	655.70 ± 103.24	479.53 ± 44.63	667.46 ± 52.21
7	846.66 ± 93.77	652.16 ± 73.22	789.86 ± 39.10
8	1109.30 ± 162.31	848.23 ± 80.36	997.38 ± 71.09
9	1298.34 ± 229.34	107.80 ± 122.73	1151.31 ± 94.75
10	1503.96 ± 242.50	1158.00 ± 134.40	1314.90 ± 125.27

Table 3. Mean and standard deviation Smad-3 MMP-2 gene in study groups

Group Variable	Intensity interval training	Aerobic continuous training	Control
Smad-3	1.454 ± 0.323	1.455 ± 0.451	1
MMP-2	0.577 ± 0.75	0.737 ± 0.279	1

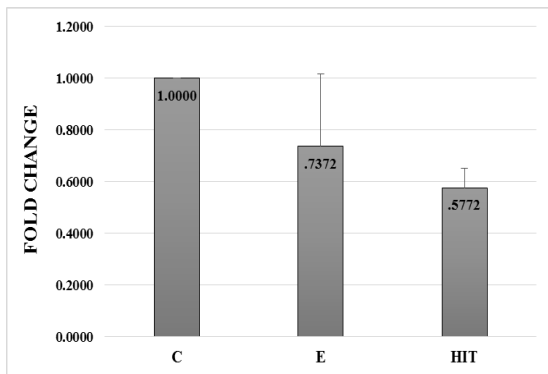


Figure 1. Change in expression of MMP-2 after the intervention stage

Also, there were significant differences in intensity interval training group and control group, continuous endurance group and control group and between intensity intermittent and continuous endurance groups in the amount of TGF-β (P < 0.05) (Figure 2).

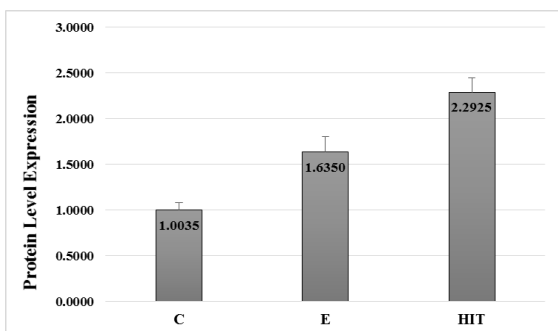


Figure 2. TGF-B protein values after the intervention stage

In addition, there was significant differences between the two groups of high-intensity intermittent exercise and control group, as well as continuous endurance group and control group in Smad-3 gene expression (P < 0.05). But no significant differences between training groups in the Smad-3 gene expression was observed (P > 0.05) (Figure 3).

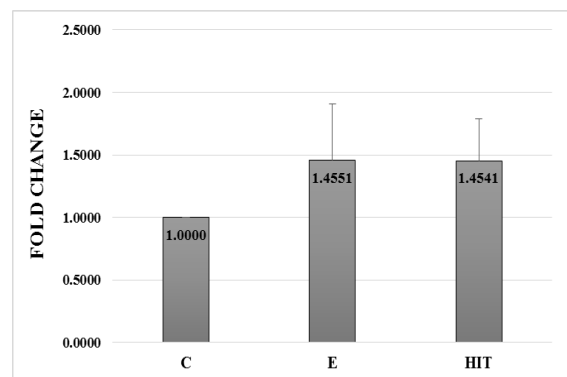


Figure 3. Changes in the expression of Smad-3 after the intervention stage

Conclusion:

The results of the current research showed that high intensity intermittent aerobic exercise significantly decreased the mean value of expression of MMP-2 in tumor tissue. Studies have shown the possibility of the influence of MMP-2 in physical exercises. In this regard, some research has reported the reduction of MMP-2 after physical

activity. Consistent with the findings of this study, Seidanloo and Farzanegi concluded that regular physical activity (Pilates exercises for eight weeks, three sessions per week, and each session 60 minutes) in overweight women, significantly reduces the amount of MMP-2. Their results showed that Pilates training has a positive effect on MMP-2 in overweight women (22). Also, Posa et al (2015) study showed that physical activity is optional six weeks reduced serum levels of MMP-2 in desert rats (23). Changes in serum levels of MMPs are as a result of changes in the MMPs in tissue (28). TIMPs and $\alpha 2$ Microglobulin and TSP-1 are the most important inhibitors of MMPs (29). Rullman and colleagues demonstrated an increased amount of MMPs mRNA immediately after the exercise. They stated that these factors may cause reduction of MMP-2 in response to exercise (28). $\alpha 2$ microglobulin is the largest anti-serum protease which is made by the liver macrophages and fibroblasts and influences almost all of proteinase, regardless of their stages. $\alpha 2$ -microglobulin binds only to activate MMPs (30). Ferguson and colleagues observed the increase in $\alpha 2$ -microglobulin levels after an exercise period (31). TSP-1 also through the connection of MMP-2 zymogens prevents activation of the MMP-2. Therefore, TSP-1 reduces the level of MMP-2 activity (32). Research has shown that the level of TSP-1 increases in response to physical activity (33). On the other hand, glycoprotein receptors called integrins, are the main binders of extracellular matrix ligands and cytoskeleton structures. Research has shown that MMPs are able to connect to these receptors, therefore the binding of MMP-2 to integrin receptors can also reduce MMP-2, and is one of the factors (34). Some studies have also reported no change or no increase of MMP-2 after physical activity (34-36). The reasons for the differences in these results with previous studies could be related to the intensity and duration of activity so that after protocol and preparation of increased short-term (less than 20 min), no change in MMP-2 have been reported (36). Also in response to an exhaustive aerobic exercise, significantly increased levels of MMP-2 have been reported (37). The reason for this difference may be as a result of more severe damage to the myofibrils of muscles caused by

activities involved in running on a slope that could result from further changes in the levels of MMPs while the study was conducted about the vigorous aerobic continuous and intermittent exercises. Also, in a study, after cycling in the form of periodic and high-intensity cycling in 10 three-minute periods, about 85% of a significant increase in serum levels of MMP-2 was reported (38). The results of the above study are inconsistent with current research, and appear to be Continuation of iterations and, a lot of periodicity in this area was effective and caused discrepancies in results.

According to the methods employed in this study such kind of training, participants, as well as sampling (muscle biopsy) with the current study, has a significant difference. Accordingly, the difference in results is conceivable. On the other hand, in spite of much research about finding markers for tracking the status of published tumors seems that scholars' debate and struggle over this issue persists. And they still have not reached a consensus agreement on such markers. And a combination of multiple markers should be given to increase the specificity and sensitivity of this issue (39). The results of this study show that high-intensity intermittent aerobic exercise and caused a significant increase in a mean value of TGF- β in protein concentration in tumor tissue in practice groups. According to the analysis done, this is the first study that levels of β -TGF subsequent periodic training and endurance are reported in terms of breast cancer. The results of the current research were in line studies in which increased levels of TGF- $\beta 1$ after acute and long-term activities have been reported (20,40). The pattern of changes in serum levels of TGF- $\beta 1$ expression, muscle and tissue are quite different which confirms that the serum levels of TGF- $\beta 1$ protein expression are not affected by muscle and tissue. In previous research, increased expression of TGF- $\beta 1$ has been reported in various tissues after acute exercise as well (41).

Another factor is increasing blood lactate levels and muscles that are an integral part of intense interval training. Lactate positive effects on raising the expression of TGF- $\beta 1$ in the cerebrospinal fluid have been reported in previous studies (42). With the rise in blood lactate levels during exercise and muscle HITT, the substrate that can be easily cross the blood brain barrier, and rise lactate in the brain

and increase expression of TGF- β 1 is the ultimate outcome of this process is a rise of the expression of TGF- β 1 in blood and tissues. The rise of TGF- β 1 is uncertain, however, oxidative stress and hypoxia induced by exercise are two possible mechanisms (18). Studies showed that by increasing expression of NADPH oxidase and decreased expression of superoxide dismutase raises expression of TGF- β 1 in cardiac muscle and kidney (43). Because after an intense workout the situation of oxidant/antioxidant of muscle changes, according to the antioxidant effects of TGF- β 1 probably one of the reasons for the increase of TGF- β 1 after training is to support the antioxidant status. The other more important reason, is the occurrence of cellular hypoxia as a result of HIT training. Doing these exercises accompanies with severe hypoxia in muscle cells, which leads to increased expression of HIF-1 α (Hypoxia-induced Factor 1- α). This factor is increased in cells in response to hypoxia. And it mediates the biological activities of numerous intermediaries. In previous research, increased expression of TGF- β 1 influenced by the increased expression of HIF-1 α has been reported (44), which can be upregulated HIF-1 α as a factor influencing the expression of TGF- β 1 after intense workouts raise. In support of this possibility, the increase in mRNA β 1 TGF content immediately after endurance training has been reported in previous studies (45). The TGF- β 1 is a multifunctional protein that acts as an anti-inflammatory cytokine as well (45). Due to the fact that severe sports activities cause destruction of fiber and inflammatory responses associated with it, and another reason for the increased expression of TGF- β 1 may be caused by inflammation of muscle tissue after intense training. However, some research has been reported the reduction of serum levels of TGF- β as a result of exercise (19,46). Perhaps decreased levels of TGF- β is followed by training is influenced by the type of training. Because the levels of TGF- β acts against hypertrophy exercise and stimulates atrophy, as a result, its serum level is decreased. It has been shown that eight weeks of swimming training leads to no changes in the levels of TGF- β in healthy rats (47). Exercise protocol in the above study, taking into account the induction of diabetes, from 5 minutes swimming in the first week rose to 30

minutes swimming in the third week and then continued for 8 weeks. It seems that the above exercise did not induce enough adaptations in healthy mice, therefore, was not able to change the indices of cardiac tissues. This is probably related to insufficient intensity and duration of exercise or normal adjustment of indicators in the heart tissue of diabetic rats compared to the healthy ones. At the most, the results of a current study indicate that high intensity intermittent aerobic exercise has significantly increased a mean value of expression of Smad-3 in tumor tissue in the endurance group with respect to the control group. Research have shown that TGF- β signaling pathway starts with stimulation of Smad2 and Smad3, and finally, under the influence of Smad4 receiver serine-threonine kinase is connected to the cell phosphorylation membrane and is activated (48).

This protein is in the form of identical or non-identical compounds with other members of the Smad family enters into the core and does the process of prohibiting cell differentiation and cell growth arrest (49). The main objective of TGF- β /Smad-3 is Non-proliferation and cell growth process (50). Therefore, he increases the expression of Smad-3/TGF- β as a result of endurance training can affect the rise in the inhibition of this pathway and can prevent cell differentiation and cell growth in the tumor. Unfortunately, the study method is not the way that can be certainly commented about this factor. The further study with simultaneous control of several factors is recommended to further researchers. The observed increase in the expression of TGF- β and Smad-3 in the present study seeks to apply cardio and interval, by considering the role of these factors in cell differentiation and growth inhibition of tumor cells prohibition, this factor has special importance. However, in this study, levels of MMPs and other factors of Smads not measured. In addition, the lack of control over the strict diet (measured energy intake and consumption) is one of the limitations of this study. Finally, according to the results suggest that the check the strength training on inflammatory responses in breast cancer. It is also recommended that the above cases involving human subjects.

In conclusion, according to the findings of the study, it showed that aerobic training and periodic significantly decreased expression of MMP-2 and

significantly increased a mean value of protein concentration TGF- β and Smad-3 gene expression in tissue. According to the findings of current research, it seems that aerobic endurance and high-intensity intermittent training may be effective interventions in reducing breast cancer progression, and there is not much difference between the effects of two types of training.

Acknowledgment:

This field of research was carried out in Central Tehran Branch, Islamic Azad University. Hereby, the authors Thank and appreciate of this unit of university.

References:

- Boyle P, Levin B. World Cancer Report Geneva. Iyon: WHO Press; IARC Publication. 2008.
- Hasanpur DA, Azari S. Quality of life related factor in cancer patients. *Behbood*. 2006;10(2):110-119.
- Cianfrocca M, Goldstein LJ. Prognostic and Predictive Factors in Early-Stage Breast Cancer. *Oncologist*. 2004;9(6):606-616.
- Jurasz P, Sawicki G, Duszyk M, Sawicka J, Miranda C, Mayers I, et al. Matrix metalloproteinase 2 in tumor cell-induced platelet aggregation: regulation by nitric oxide. *Cancer Res*. 2001;61(1):376-382.
- Koskinen SO, Höyhty M, Turpeenniemi-Hujanen T, Martikkala V, Mäkinen TT, Oksa J, et al. Serum concentrations of collagen degrading enzymes and their inhibitors after downhill running. *Scand J Med Sci Sports*. 2001;11(1):9-15.
- Reiss M, Barcellos-Hoff MH. Transforming growth factor-beta in breast cancer: a working hypothesis. *Breast Cancer Res Treat*. 1997;45(1):81-95.
- Derynck R, Akhurst RJ, Balmain A. TGF-beta signaling in tumor suppression and cancer progression. *Nat Genet*. 2001;29(2):117-129.
- Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF- β family signalling. *Nature*. 2003;425(6958):577-584.
- Moustakas A, Heldin P. TGF β and matrix-regulated epithelial to mesenchymal transition. *Biochim Biophys Acta*. 2014;1840(8):2621-2634.
- Khosropanah MH, Dinarvand A, Nezhadhosseini A, Haghighi A, Hashemi S, Nirouza F, Khatamsaz S, Entezari M, Hashemi M, Deghani H. Analysis of the Antiproliferative Effects of Curcumin and Nanocurcumin in MDA-MB231 as a Breast Cancer Cell Line. *Iran J Pharm Res*. 2016;15(1):231-239.
- Kalalinia F, Elahian F, Mosaffa F, Behravan J. Celecoxib Up Regulates the Expression of Drug Efflux Transporter ABCG2 in Breast Cancer Cell Lines. *Iran J Pharm Res*. 2014;13(4):1393-1401.
- Ligibel JA, Giobbie-Hurder A, Olenczuk D, Campbell N, Salinardi T, Winer EP, et al. Impact of a mixed strength and endurance exercise intervention on levels of adiponectin, high molecular weight adiponectin and leptin in breast cancer survivors. *Cancer Causes & Control*. 2009;20(8):1523-1528.
- Westerlind KC, McCarty H, Schultheiss P, Story R, Reed A, Baier M, et al. Moderate exercise training slows mammary tumour growth in adolescent rats. *European Journal of Cancer Prevention*. 2003;12(4):281-287.
- Lee I-M. Physical activity and cancer prevention-data from epidemiologic studies. *Medicine and Science in Sports and Exercise*. 2003;35(11):1823-1827.
- Donley DA, Fournier SB, Reger BL, DeVallance E, Bonner DE, Olfert IM, et al. Aerobic exercise training reduces arterial stiffness in metabolic syndrome. *J Appl Physiol*. 2014;116(11):1396-1404.
- Cheema BS, Gaul CA. Full-body exercise training improves fitness and quality of life in survivors of breast cancer. *Journal of Strength and Conditioning Research*. 2006;20(1):14-21.
- Leite RD, Durigan Rde C, de Souza Lino AD, de Souza Campos MV, Souza Md, Selistre-de Araújo HS, et al. Resistance training may concomitantly benefit body composition, blood pressure and muscle MMP-2 activity on the

- left ventricle of high-fat fed diet rats. *Metabolism*. 2013;62(10):1477-1484.
18. Sajadian S, Nikooie R. TGF- β 1 protein Expression in the Skeletal Muscle Following High Interval Training and its Relationship with Intramuscular Triglycerides Oxidation. *Journal of Sport in Biomotor Sciences*. 2015;6(12):45-54. [Persian]
 19. Khadivi BA, Marandi M, Haghjooy JS, Rajabi H, Khadivi BZ, Khorshidi BM. Effect of Eight Weeks of Resistance Training on Some Signaling Factors Affecting on the Satellite Cells in Wistar Rats. *Journal of Isfahan Medical School*. 2012;30(207)1-12.
 20. Czarkow SKA-Paczek B, Bartlomiejczyk I, Przybylski J. The serum levels of growth factors: POGF, TGF-BETA and VEGF are increased after strenuous physical exercise. *Journal Physiology and Pharmacology*. 2006;57(2):189-197.
 21. Santos AR, Neves MT Jr, Gualano B, Laurentino GC, Lancha AH Jr, Ugrinowitsch C, et al. Blood flow restricted resistance training attenuates myostatin gene expression in a patient with inclusion body myositis. *Biol Sport*. 2014;31(2):121-124.
 22. Seidanloo F, Farzanegi P. Changes in Matrix Metallo-proteinases 2, 9 and Tissue Inhibitor of Matrix Metalloproteinase 1 to Synchronized Exercise Training and Celery, as an Herbal Supplement, in Overweight Women. *Modares Journal of Medical Sciences: Pathobiology*. 2016;1(18):107-118. [Persian]
 23. Pósa A, Szabó R, Kupai K, Baráth Z, Szalai Z, Csonka A, et al. Cardioprotective Effects of Voluntary Exercise in a Rat Model: Role of Matrix Metalloproteinase-2. *Oxidative Medicine and Cellular Longevity*. 2015;876805-9.
 24. Amani-shalamzari S, Agha-Alinejad H, Alizadeh S, Shahbazi S, Khatib ZK, Kazemi A, et al. The effect of exercise training on the level of tissue IL-6 and vascular endothelial growth factor in breast cancer bearing mice. *Iranian Journal of Basic Medical Sciences*. 2014;17(4):231-236.
 25. Jones LW, Viglianti BL, Tashjian JA, Kothadia SM, Keir ST, Freedland SJ, et al. Effect of aerobic exercise on tumor physiology in an animal model of human breast cancer. *Journal of Applied Physiology*. 2010;108(2):343-348.
 26. Hassanzadeh K, Nikzaban M, Moloudi MR, Izadpanah E. Effect of selegiline on neural stem cells differentiation: a possible role for neurotrophic factors. *Iran J Basic Med Sci*. 2015;18(6):549-554.
 27. Rahimmi A, Khosrobakhsh F, Izadpanah E, Moloudi MR, Hassanzadeh K. N-acetylcysteine prevents rotenone-induced Parkinson's disease in rat: An investigation into the interaction of parkin and Drp1 proteins. *Brain Res Bull*. 2015;113:34-40.
 28. Rullman E, Wagsater D, Fischer H, Eriksson P, Sundberg CJ, Jansson E, et al. A single bout of exercise activates matrix metalloproteinase in human skeletal muscle. *J Appl Physiol*. 2007;102(6):2346-2351.
 29. Moses MA. The Regulation of Revascularization by Matrix Metalloproteinase and Their Inhibitors. *Stem Cells*. 1997;5(3):180-189.
 30. Ferguson EW, Bernier LI, Banta GR. Effect of exercise and conditioning on clotting and fibrinolytic activity in men. *J Appl Physiol*. 1987;62(4):1416-1421.
 31. Bein K, Simons M. Thrombospondin type 1 repeats interact with matrix metalloproteinase 2. Regulation of metalloproteinase activity. *J Biol Chem*. 2000;275(41):32167-32173.
 32. Olfert IM, Breen EC, Gavin TP, Wagner PD. Temporal mRNA thrombospondin-1 response in skeletal muscle exposed to acute and chronic exercise. *Growth Factors*. 2006;24(4):253-259.
 33. Silletti S, Kessler T, Goldberg G, Boger DL, Cheresch DA. Disruption of matrix metalloproteinase 2 binding to integrin $\alpha\beta$ 3 by an organic molecule inhibits angiogenesis and tumor growth in vivo. *PNAS USA*. 2001;98(1):119-124.
 34. Mackey AL, Donnelly AE, Swanton A, Murray F, Turpeenniemi-Hujanen T. The effects of impact and non-impact exercise on circulating markers of collagen remodelling in humans. *Sports Sci*. 2006;24(8):843-848.
 35. Nazari M, Kordi MR, Choobineh S. The Effect of High Intensity Interval Training (HIIT) on Gelatinase-A (MMP-2) Serum

- Levels and Muscle Damage Indices in Young Sedentary Girls. *Arak University of Medical Sciences Journal*. 2015;18(94):78-86. [Persian]
36. Danzig V, Mikova B, Kuchynka P, Benakova H, Zima T, Kittnar O, et al. Levels of circulating biomarkers at Rest and after exercise in coronary artery disease patients. *Physiological Research*. 2010;59:1374-1379.
37. Amirsasan R, Mirshafiei A, Gaeini AA, Ravaasi AA, Letafatkar A, Saadat F, et al. Effects of Exhaustive Aerobic Exercise on Matrix Metalloproteases. Activity in Athletes and Non-Athletes. *World Journal of Sport Sciences*. 2011;4(2):185-191.
38. Suhr F, Brixius K, de Marées M, Bölc B, Kleinöder H, Achtzehn S, et al. Effects of short-term vibration and hypoxia during highintensity cycling exercise on circulating levels of angiogenic regulators in humans. *Journal of Applied Physiology*. 2007;103(2):474-483.
39. Lumachi F, Brandes AA, Boccagni P, Polistina F, Favia G, Amico DF. Long-term follow-up study in breast cancer patients using serum tumor markers CEA and CA 15-3. *Anticancer Res*. 1999;19(5):4485-4490.
40. Hering C, Jost H, Schulz B, Hellmich H, Schatz A, Pfeiffer A. Circulating transforming growthfactor fl1 (TGFB1) is elevatedin extensive exercise. *J Appl Physiol*. 2002;86(5):406-441.
41. Kimsa M, Strzalka-Mrozik B, Kimsa M, Gola J, Kochanska-Dziurawicz A, Zebrowska A, et al. Expression pattern of the transforming growth factor β signaling genes in human peripheral blood mononuclear cells after exercise-inflammatory aspects. *American Journal of Human Biology*. 2012;24(6):859-862.
42. Yamda H, IwakiY, Kitaoka R, Fujitani M, Shibakusa T, Fujikawa T. Blood Lactate Functions as a Signal for Enhancing Fatty Acid Metabolism during Exercise via TGF- β in the Brain. *J Nutr Sci Vitaminol*. 2012;58(2):88-95.
43. Zhao W, Zhao T, Chen Y, Ahokas RA, Sun Y. Oxidative stress mediates cardiac fibrosis by enhancing transforming growth factor-beta1 in hypertensive rats. *Mol Cell Biochem*. 2008;317(1-2):43-50.
44. Milkiewicz M, Doyle JL, Fudalewski T, Ispanovic E, Aghasi M, Haas TL. HIF-1 α and HIF-2 α play a central role instrech-induced but not shear-stress-induced angiogenesis inrat skeletal muscle. *J Physiol*. 2007;583(Pt2):753-766.
45. Czarkowska B, Zendzian M, Bartlomiejczyk I, Przybylski J, Gorski J. The effect of acute and prolonged endurance exercise on transforming growth factor-beta1 generation in rat skeletal and heart muscle. *J Physiol Pharmacol*. 2009;60(4):157-162.
46. Feinberg MW, Jain MK, Werner F, Sibinga NE, Wiesel P, Wang H, et al. Transforming growth factor-beta 1 inhibits cytokine-mediated induction of human metalloelastase in macrophages. *J Biol Chem*. 2000;275(33):25766-25773.
47. Habibian M, Khosravi M. The Effect of 8 weeks regular swimming exercise on the cardiac levels of matrix mettaloproteinase-2 and transforming growth factor-B1 in diabetic rats. *Ijldl*. 2016;15(2):67-74. [Persia]
48. Moustakas A, Souchelnytskyi S, Heldin CH. Smad regulation in TGF-beta signal transduction. *J Cell Sci*. 2001;114(Pt24):4359-4369.
49. Glass DJ. Signaling pathways perturbing muscle mass. *Curr Opin Clin Nutr Metab Care*. 2010;13:225-229.
50. Braga M, Bhasin S, Jasuja R, Pervin S, Singh R. Testosterone inhibits transforming growth factor- β signaling during myogenic differentiation and proliferation of mouse satellite cells: Potential role of follistatin in mediating testosterone action. *Mol Cell Endocrinol March*. 2012;350(1):39-52.