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The effect of physical fitness level on mir-155 gene expression mediated by job stress in Shiraz firefighters

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ABSTRACT

Introduction: Firefighters are increasingly exposed to factors that affect their physical and mental health. The aim of present study was examined the effect of physical fitness level on mir-155 gene expression mediated by job stress in Shiraz firefighters.

Material & Methods: Twenty firefighters volunteered to participate in this study. To measure the physical fitness of the subjects, the Physical Fitness Index Questionnaire (Sharkey, 1990) was used, and to assess the level of job stress, the Job Stress Questionnaire (Elliott, 1994) was used. The expression of the mir-155 gene was also measured by Real-time PCR. Smart PLS software was used to analyze the data, with a significance level of $p<0.05$.

Results: The results showed that the level of physical fitness and job stress did not have a direct and significant effect on the expression of the mir-155 gene in Shiraz firefighters, and the level of physical fitness also did not have a direct and significant effect on their job stress.

Conclusion: These results indicate that to improve the health and performance of firefighters, attention should be paid to other factors that may have a greater impact on gene expression and job stress.

Keywords: Physical fitness, Job stress, mir-155, Firefighters.

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1. Introduction

Firefighting is considered a very dangerous profession that, due to the nature of its work, is often exposed to dangerous environments, emergency situations, irregular working hours, and physical and mental stress. In addition, most firefighters experience a lot of inconsistency and emotional conflict with people while performing their duties, which leads to job stress in them (1). Job-related stress may increase the risk of cardiovascular events in firefighters. Occupational risk factors for firefighters can cause physiological stress and lead to cardiovascular events (2). Also, exposure to certain chemicals, including carbon monoxide, particulate matter, and other toxic substances, and intermittent exposure to noise may increase blood pressure, with sympathetic activation, physical workload, heat exposure, and dehydration being important stressors (1). It has recently been shown that risk factors for cardiovascular disease are high among firefighters, with about 68 percent of firefighters having two or more risk factors, such as smoking, high blood pressure, obesity, high blood cholesterol, and physical inactivity. In addition, researchers stated that firefighters endure great stress on the body's physiological system, especially the cardiovascular system, during operations (3). This increases the risk of cardiovascular disease in firefighters, which worsens with age (4).

Although existing studies indicate that many firefighters have the necessary ability to perform their work duties when fighting fires, the results have shown that many of them do not have the optimal physical fitness levels required to perform their duties (5). Researchers also stated that cardiovascular health and physical fitness are factors that contribute to the occurrence of cardiovascular disease, which accounts for about 40 to 50 percent of all work-related deaths among firefighters (6). Thus, firefighters need cardiovascular health, musculoskeletal system, and an acceptable level of physical fitness to cope with the physiological and psychological stresses of work (4).

Recent genetic studies have implicated a number of miRNAs in the progression of cardiac diseases, including myocardial ischemia (7), and have clearly demonstrated the pivotal role of some miRNAs in hypertrophy, heart failure, and myocardial infarction (8). miRNAs, which are small non-coding RNAs, are considered post-transcriptional gene regulators that can regulate protein synthesis (9), and a number of miRNAs have been considered as potential biomarkers for post-myocardial infarction, among which is mir-155, whose levels are increased in patients with coronary heart disease (10). mir-155 plays a vital role in hematopoiesis, lymphocyte homeostasis, and in the cardiovascular system, mir-155 has been reported in atherosclerotic plaques and proinflammatory macrophages (11).

Based on the above, it can be said that given that recent statistics indicate the prevalence of cardiovascular disease among firefighters, work-related stress is known as one of the known cardiovascular risk factors associated with firefighters, and recent articles in a new approach have focused on the expression level of genes associated with cardiovascular diseases, but existing studies have not examined genetic factors in firefighters, so it is important to examine this issue.

2. Methodology

2.1. Materials and methods

This experimental study.

2.2. Participants

The statistical population of the present study included all firefighters of the Shiraz City Fire Department. Based on the available samples, after explaining the steps of the research design, 20 firefighters who volunteered to participate in the present design were selected. Then, the subjects completed written consent forms.

2.3. Measurements

To measure the subjects' physical fitness level, the Physical Fitness Index questionnaire was used (12). This questionnaire has three sections, the first section of which consists of 5 questions and the subject answers only one question. This section measures the intensity of daily exercise and is scored from 5 to 1. The second part of the questionnaire consists of 4 questions related to the duration of sports activity. In this part, the subject answers only one question and receives a score from 4 to 1. Finally, the third part of the questionnaire evaluates the frequency or repetition of sports activity per day, week, and month and includes 5 questions. In this section, the subject also receives a score from 5 to 1. To obtain a total score, multiply the scores of all 3 sections together and if the resulting number is higher than 100, a person has a very active life, if the score is between 60-80, a person has an active life, if the score is between 40-60, a person has acceptable physical fitness, if the score is between 20-40, a person has not very good physical fitness and finally if the score is less than 20, a person is considered inactive. Previously, the validity of this questionnaire was reported by Moghadasi et al. (2011) as 0.88 (13).

2.4. Intervention

The level of job stress was measured based on the Job Stress Questionnaire. The stress questionnaire was developed by R. S. Eliot (1994), which consists of 20 items and is used to measure the level of stress in individuals. The questionnaire is scored on a 4-point Likert scale, with scores of 1, 2, 3, and 4 for the options "never," "sometimes," "often," and "always," respectively. The reliability of this questionnaire was calculated as 0.75 in the research of Jahanbakhsh Ganja and Areezi Samani (14). To evaluate the expression of the mir-155 gene, initially 5 cc of blood was taken from the anterior vein of the subjects' hands and the expression of the mir-155 gene was measured using the Real-time PCR method. After sampling, the tubes containing peripheral blood were stored in a -80°C freezer until RNA extraction was performed. Then, to examine the level of miR-155, total RNA was extracted using a column RNA extraction kit (FavorPrep™ Tissue Total RNA Mini Kit) made in Hong Kong

Table 1. *The primer sequence used in the study*

Gene	Primer sequence
miRNA-155	Forward: TGCTAATCGTGATAGGGG Reverse: GAACATGTCTGCGTATCTC
U6	Forward: CTCGCTTCGGCAGCACAT Reverse: TTTGCGTGTCATCCTTGCG

2.5. Statistical Methods

Data analysis was performed using smart PLS software at a significance level of $p<0.05$.

3. Results

The mean and standard deviation of physical fitness, job stress, and mir-155 are shown in Table 2. The average physical fitness level of Shiraz firefighters was 51.1. According to the Physical Fitness Index questionnaire, these individuals had an acceptable level of physical fitness. The average JOB stress of Shiraz firefighters was 36.45. According to Eliot's Job Stress Questionnaire (1994), the subjects of this study had a healthy life and were in control of their lives, but sometimes they were in stressful situations and became anxious.

Table 2. *Levels of physical fitness, job stress, and mir-155 of subjects*

Variables	Mean	standard deviation
Physical fitness	51.1	17.5
Job stress	36.4	5.0
mir-155	0.95	0.6

Before examining the research hypotheses, the general research model, which includes both operational and administrative staff groups, was implemented using structural equation modeling using the PLS method. To examine the fit of the research model, the R^2 index or coefficient of determination and the t-significance coefficient were used. The coefficient of determination indicates the effect that an exogenous (independent) variable has on an endogenous (dependent) variable. In fact, R^2 examines the relationship between the variance value of a latent variable and its total variance value. It should be noted that the R^2 value is calculated for endogenous constructs of the model, and in the case of exogenous constructs, the value of this criterion is zero. The value of this coefficient is between zero and 1, with larger values being more desirable. Chain (1998) introduces three values of 0.19, 0.33, and 0.67 as criterion values for weak, moderate, and strong R^2 values. Table 3 reports the R^2 values for the endogenous variables of the study.

Table 3. *R^2 values for each of the endogenous (dependent) variables of the study*

Variables	Statistical index
	R^2
Physical fitness	0.663
Job stress	0.858

As can be seen in Table 3, both mir-155 and job stress variables have very strong R^2 values of (0.663) and (0.858) respectively. In other words, the obtained coefficients of determination show that the independent and mediator variables of the study have the ability to predict the dependent variable, i.e., mir-155 gene expression. Also, Figure 1 shows the R^2 values of each of the study variables.

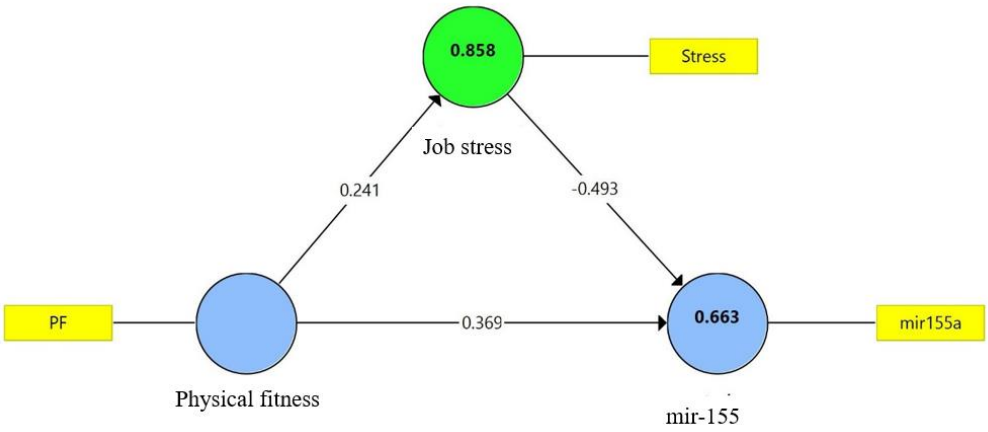

Figure 1. *Coefficients of determination and path coefficient of research variables*

In addition, the t-test shows whether the effect of the variables on each other is statistically significant at the 95% confidence level. If the t-test is greater than the criterion value, i.e. 1.96, it can it can be claimed with 95% confidence that the effect of the two variables on each other is significant. The t-coefficients are presented in Figure 2.

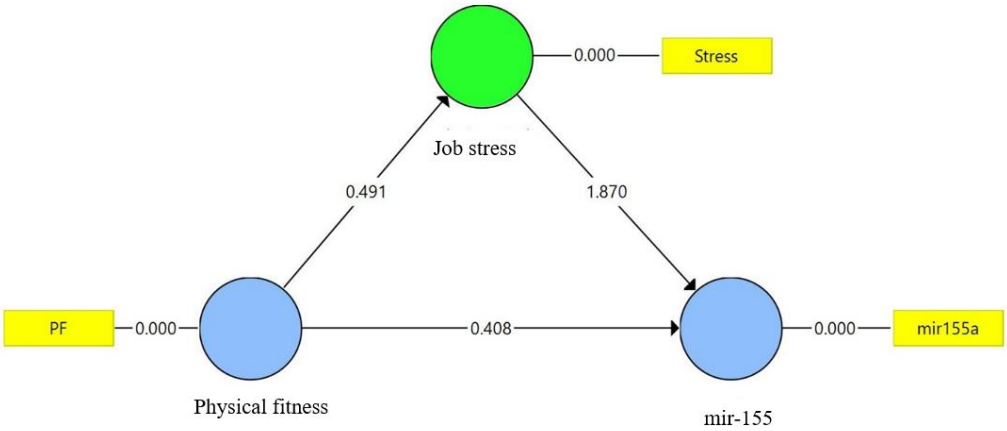

Figure 2. *T-values related to the significance of the path between research variables*

Based on the t-coefficients obtained in Figure 2, which are smaller than 1.96, it is clear that the research variables have no effect on each other.

As shown in Table 4, the significance coefficient of the path between the two variables of physical fitness level and mir-155 gene expression is equal to (0.408), which is not greater than 1.96. Therefore, it can be said that the level of physical fitness does not have a direct and significant effect on the expression of mir-155 gene in Shiraz firefighters. Also, the significance coefficient of the path between the two variables of job stress and mir-155 gene expression was obtained as (1.870), which is not greater than 1.96. Therefore, it can be said that job stress does not have a direct and significant effect on the expression of mir-155 gene in Shiraz firefighters. The significance coefficient of the path between the two variables of job stress and mir-155 gene expression was obtained as (1.870), which is not greater than 1.96. Therefore, it can be said that job stress does not have a direct and significant effect on the expression of mir-155 gene in Shiraz firefighters. Finally, the significance coefficient of the indirect path between the two variables of physical fitness level and mir-155 gene expression was obtained as (0.416), which is not greater than 1.96. Therefore, it can be said that the level of physical fitness does not have an indirect and significant effect on the expression of mir-155 gene in Shiraz firefighters through the job stress variable. In fact, the mediating role of job stress is not statistically confirmed.

Table 4. *Results of the path analysis test regarding the direct effect of variables on each other*

Path direction	Path coefficient	t	P value
Physical fitness => mir-155	0.369	0.408	0.6
Job stress => mir-155	-0.493	1.870	0.06
Physical fitness => Job stress	0.241	0.491	0.6
Physical fitness => Job stress => mir-155	-0.119	0.416	0.6

4. Discussion

The results of the present study showed that the level of physical fitness does not have a direct and significant effect on the expression of the mir-155 gene in Shiraz firefighters. Also, job stress did not have a direct and significant effect on the expression of the mir-155 gene in Shiraz firefighters. On the other hand, it was shown that the level of physical fitness does not have an indirect and significant effect on the expression of the mir-155 gene in Shiraz firefighters through the job stress variable. In fact, the mediating role of job stress is not statistically confirmed.

The results of this study are consistent with those of Mooren. (2013) (15). However, the results of the present study are not consistent with the others (6,16).

Endurance and cardiorespiratory training are an important component of increasing physical fitness levels, which is essential for firefighters. Studies have shown that exercise activities of varying intensity can lead to adaptive changes in the body's cardiovascular system. These adaptations include increased myocardial angiogenesis, increased coronary blood flow, and reduced inflammatory response in the body (17). Meza et al. (2022) were studied the effect of eight weeks of combined exercise training on circulating microRNAs in young, healthy men (18). They indicated that eight weeks of combined exercise training do not alter circulating microRNAs-29a, -133a, -133b, and -155. It is worth noting, however, that no relevant study was found on the role of mir-155 in firefighter subjects. However, regarding the role of exercise on mir-155 levels, in a study by Li et al. (2021), mir-155 levels were examined in young individuals before and after aerobic exercise training (5 km continuous running). The results showed that compared to the resting state, serum mir-155 levels increased significantly after exercise training. Serum levels of mir-155 after exercise training were also positively correlated with creatine kinase and lactate dehydrogenase. These researchers stated that dynamic changes in miRNAs under aerobic exercise conditions help regulate adaptations in cardiovascular function (19).

Regarding the physical fitness levels of firefighters and cardiovascular disease, Ros et al. (2023) showed that there is a significant and inverse relationship between cardiovascular disease risk factors and cardiorespiratory fitness (20). Based on the results obtained, these researchers stated that fire departments should adopt behavioral intervention strategies to maintain optimal indices of cardiovascular disease risk factors and cardiorespiratory fitness among firefighters to ensure their occupational well-being. However, the present study showed that the level of physical fitness does not have a direct, indirect, or significant effect on the expression of the mir-155 gene (as an indicator for examining cardiovascular health in firefighters) and the job stress of firefighters. This difference in results may be due to differences in the variables examined and our research methods compared to those of Ras et al. While Ras's research focused on the relationship between cardiorespiratory fitness and cardiovascular disease risk factors, our study examined the effect of physical fitness on mir-155 gene expression as a factor associated with firefighters' cardiovascular disease susceptibility and psychological job stress.

Firefighting increases the risk of developing numerous physiological disorders, including cardiovascular, musculoskeletal, psychiatric, and other diseases. The job of a firefighter is physically demanding and involves activities such as running, climbing, pulling, and lifting. Often, these tasks are unpredictable, performed in harsh environments, and have been shown to cause psychological stress (20). In a study, Soteriades et al. (2022) examined the impact of exercise activity, physical fitness levels, and job stress among firefighters (21). The results showed that each hour of weekly increase in physical activity among firefighters was associated with a 16 percent reduced risk of job stress. Furthermore, the researchers' findings suggest that there is an inverse dose-response relationship between physical activity and job stress among firefighters.

These researchers stated that physical activity appears to be inversely related to job stress and acts as an important factor in reducing job stress in firefighters. The results of the study by Soteriades et al. (2022) are not consistent with the results of the present study; because in the present study, it was observed that the level of physical fitness does not have a direct and significant effect on the job stress of firefighters in Shiraz, while in the study by Soteriades et al. (2022), job stress decreased following an increase in physical activity among firefighters (21).

5. Conclusion

Generally, the results showed that the level of physical fitness and job stress did not have a direct and significant effect on the expression of the mir-155 gene in Shiraz firefighters. Also, the level of physical fitness does not have a direct and significant effect on job stress, and its indirect effect on mir-155 gene expression through job stress is also not significant. These results may be due to other factors that were not examined in this study, such as nutrition, genetics, or environmental conditions, etc. Also, firefighters' bodies may be regulated in such a way that these factors do not have a significant effect on mir-155 gene expression due to physiological adaptation to job stresses. To better understand these relationships, it is suggested that further research should be conducted by considering various other factors. These results suggest that to improve the health and performance of firefighters, other factors that may have a greater impact on gene expression and job stress should be considered.

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Conflict of interests: The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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