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Heart and stress: A morphometric and light microscopic study in a rat model

Tuba DEMIRCI[1], Elvan SAHIN[2]

ABSTRACT

Background: Stress increases the risk of cardiac diseases, including heart attack and sudden death. In this study, we aimed to investigate the histological changes of the myocardium of stress-exposed rats. **Material and methods:** Twelve-male rats were randomly divided into control (n=6) and stress groups (n=6). Chronic mild stress procedure was applied to the rats of stress group during four weeks. At the end of the experiment, rats were sacrificed under anesthesia and blood samples were taken for assessments of serum cortisol level. Subsequently, the hearts were removed and the volume of each heart was measured by using water immersion method. After routine histologic procedure, sections were stained with Haematoxylin-Eosin and evaluated under the light microscope. **Conclusion:** The volumes of hearts of the stress group were found significantly increased when compared to the control group ($p<0.05$; Mann-Whitney U Test). Pericellular oedema, cytoplasmic swelling and necrotic degenerations of cardiac muscle cells were observed in the sections of the stress-exposed rats. Also, myocardial blood vessels were enlarged. Additionally, it was noticeable that there were prominent oedema, mononuclear cell infiltration and many adipocytes in perivascular areas. According to our findings, it was concluded that chronic stress could cause cardiac damage by adversely affecting normal histological structure of the heart muscle.

Key words: Cardiomyocyte damage; chronic stress; histopathology

Introduction

Stress is an unpleasant state that changes physiological homeostasis and the balance of organisms (1) and leads to long-term pathological changes (2). Stress effects many systems and may be an initial factor that can affect almost all of the biological functions of humans (3). For example; digestive tract, circulatory and neuroendocrine diseases (1), chronic anxiety, depression, obesity, immunologic disorders, inflammation, insulin resistance and cardiovascular diseases can occur during chronic stress and stressful life events (2). Many stressors may enhance risk of cardiovascular disease, ischemic heart disease or an important coronary event (4). Laboratory models of mental stress have shown that there is an association between stress and myocardial ischemia. Mental stress leads to left ventricular dysfunction, regional wall motion, and perfusion abnormalities in the majority of patients with coronary artery disease (5). Both mental stress and physical stress can cause myocardial ischemia. But, mental stress induced myocardial ischemia is not associated to severity of coronary atherosclerosis and cardiovascular responses caused by mental stress are completely different from those stimulated by physical effort; and signs of ischemia such as chest pain and electrocardiographic changes are less seen. The possibility of unfavourable cardiac events and general mortality are more than 2 times in the mental stress induced myocardial ischemia (6, 7). Mental stress is a common condition in the patients with cardiac diseases and it results in a series of responses that are initiated by activation of the hypothalamic–pituitary–adrenal axis (HPA axis) and organized by the adrenocorticotrophic hormones (7). The hypothalamic–pituitary–adrenal axis (HPA axis) is activated by stress. The increased cortisone level in the blood, elevated sympathetic tone and high circulation levels of catecholamine are indicators for the activation of the HPA axis (1-3, 8). It is known that chronic stress induces regional adrenal hypertrophy (9) and hyperplasia (10).

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[1]Assistant Professor of Histology-Embryology, Department of Histology and Embryology, Medical School, Atatürk University, 25240, Erzurum, TURKEY
[2]Research Unit Professor of Histology-Embryology, Department of Histology and Embryology, Medical School, Sakarya University, Sakarya, TURKEY

Corresponding author: Dr. Tuba Demirci
drtubademirci@hotmail.com
Tel : +90 505 7136344

Psychosocial stress triggers a wide variety of cardiovascular pathologies including cardiac hypertrophy, arrhythmia, heart rate variation, left ventricular systolic and diastolic dysfunction, ischemia injury and myocardial infarction (2, 3, 8). Chronic social stress may increase the risk of developing hypertension in animals (11). Chronic hypertension may play a negative role on the myocardial response to ischemia. Chronic stress constitutes a higher risk of lethal arrhythmias and contractile failure in the hearts of normotensive individuals exposed to an acute ischemic state (12). Depression is an independent risk factor for cardiovascular diseases as myocardial infarction. Chronic stress leads to depression and excessive reaction to stressors, and causes disorders of cardiac function such as elevated heart rate, reduced heart rate variability, elevated sympathetic activity in rats (13). The chronic mild stress (CMS) model, developed by Willner (14), is a quite acceptable and valid model and is frequently used to create an animal model of depression by applying various restrictions to the animals. CMS procedure includes many different stressors applied to the animals in a prolonged period. As a result of the application, the animals are reluctant to sucrose intake, and are developed a depressive state called anhedonia (15-17). The present study was designed to observe the possible hazardous effects of CMS on the heart of stress-exposed rats by performing histopathologic evaluation.

Materials and Methods

Animals

Twelve adult male Sprague Dawley rats were used in this study. Rats were housed in a room with a 12 h light–dark cycle. They were fed a standard rat chow and had free access to tap water. Throughout the experiment (4 weeks), body weights and body lengths of all subjects were measured weekly and the results were recorded regularly and then body mass index (BMI=kg/m^2) was calculated. The experimental procedure used in this study was conducted in accordance with the guidelines of the Board of Ethics in Animal Experiments.

CMS Procedure

Twelve rats were randomly divided into two equal groups, as a control (CG) and a stress (SG) group. Rats in each group were kept as 6 for one cage.

The CMS procedure was performed on the SG during the four weeks. Rats of the CG were not applied the stress procedure. In the CMS procedure (14-16, 18, 19), the stressors such as kept in a wooden box, exposing to the 240 Hz noise, put in a separate cage, swimming, applying a tiny electric shock were applied.

Detection of serum cortisol levels

At the end of the experimental procedure, rats were anesthetized with ketamine HCl (50 mg/kg, Ketalar®, Pfizer, Turkey) and xylazine (10 mg/kg, Rompun®, Bayer, Canada) and the abdomen was opened quickly by abdominal incision. Blood samples were taken from the abdominal aorta and serum was separated for biochemical assessment of cortisol. Serum samples were stored at -80 °C until assay. On the day of analysis, samples were removed from -80 °C and the levels of serum cortisol were detected by chemiluminometric assay (Immulite 2000, Siemens Inc., Deerfield, IL, USA).

Cardiac morphometry

Under ketamine HCl and xylazine anesthesia the hearts of the subjects were rapidly removed after opening their chest cages. Then, the each of the hearts was sunk in 10% formalin solution, and the volume of the heart was measured using the water immersion method "WIM" as described before (20).

Histopathological examination

After the heart volume estimation, the samples were fixed in the 10% formalin solution for 48-55 hours, dehydrated in graded alcohol series, embedded in paraffin wax, and cut using a Leica RM2125RT microtome (Leica, Germany). Paraffin sections of 5-µm thickness were stained with Hematoxylin and Eosin (H&E) and examined under a Nikon Eclipse E600 light microscope (Nikon, Japan) equipped with a digital colour camera attachment (Nikon DS-Fi1, Japan).

Statistical analysis

The Mann-Whitney U test was used for the comparisons of differences between the groups. A p value of less than 0.05 was considered significant.

Results

The effect of stress on BMI

The mean BMIs of the rats at 0 and at 4 weeks are shown in Figure 1. To investigate the effects of stress on the body weights of the rats, the BMIs of all rats were calculated at 4-week of the experiment and the values of SG were compared with the CG. When the mean BMIs of SG is compared with the CG, there was not a statistically significant

difference between the groups (p>0.05; Mann-Whitney U Test).

The effect of stress on serum cortisol levels

Serum cortisol levels of the both groups are shown Table 1. Cortisol levels measured in the serum of the SG rats was significantly higher when compared to the control group (p<0.05; Mann-Whitney U Test).

The effect of stress on cardiac morphometry

The volumes of the heart of the CG and SG measured using the WIM are shown in Table 1. The mean heart volume of the CG was 1.10 ± 0.13 ml and the one of SG was 1.33±0.12 ml. The volumes of the hearts that belong to the stress performed rats were significantly increased when compared to the control group (p=0.018; Mann-Whitney U Test).

Histopathological examination

The hearts were cut as longitudinal and whole cardiac areas were examined for histological analysis. In the histologic sections of ventricular myocardium of the CG hearts, there were no histopathologic findings (Figure 2a). But, a disruption of myocardial architecture was present in SG hearts (Figure 2b, 2c, 3a, 3b). Cytoplasmic swelling (Figure 2b) and extensive vacuolar degeneration (Figure 2b, 3b) were seen in cardiac muscle fibres of SG. The presence of many adipocytes in the some essels (Figure 2c) and within the some perivascular areas of SG hearts was noteworthy (Figure 3a). Apart from these, perivascular oedema and mononuclear cell infiltrations were observed in SG myocardium (Figure 3a). Some cardiac muscle cells had a dark and shrunken nucleus and dense eosinophilic cytoplasm. Mononuclear cell infiltrations and interstitial oedema around these degenerative and necrotic myocardial cells of SG hearts were striking (Figure 3a, 3b).

Discussion

The present study has focused on examining the effects of CMS induced by different stressors on cardiac muscle. Psychosocial factors like depression, anxiety, personality factors and character traits, social isolation and chronic life stress play an important role in the occurrence of coronary artery disease (21). Another study has reported that early maternal separation could lead to myocardial damage in rats (22).

Some researchers have reported that the increase in the blood pressure caused by chronic psychosocial stress continued for months despite the removal of the social stimuli, and there was an important development of aortic arteriosclerosis and myocardial fibrosis (23). Nevertheless, sympathetic mechanisms may be more significant in the early phases of stress-induced hypertension (24).

There are significant links between mental stress and myocardial ischemia, and the cause of myocardial ischemia in 30-70% of the patients with coronary artery disease can be acute mental stress (25). Acute emotional stress leads 40 to 60% of the deaths due to cardiac causes (26).

One of the psychosocial factors that affect the onset and course of coronary heart diseases is stress (27).

At the same time, stress can cause cardiomyopathy by affecting the myocardial layer (28).

However, there is an association between the chronic stressful states such as work stress, marital stress, low socioeconomic status and cardiovascular diseases as coronary artery disease and other adverse cardiac events. There is a neurohormonal regulation in the myocardium and disruption of this regulation may lead to serious conditions. Also, stressful life events such as acute social stress, sentimental distress, and lack of sleep may cause a number of important cardiovascular diseases. Recent studies have shown that mental stress-induced temporary cardiac dysfunction is common in the patients with ischemic hearth disease and has noteworthy clinical consequences (4).

After the CMS procedure, we observed that there is myocardial deterioration in the cardiac sections of the stress-exposed rats. In our study, we found that there were cytoplasmic swelling, extensive vacuolar degeneration and expansion of blood vessels. It has been stated that chronic stress causes myocardial swelling, interstitial vascular dilatation, congestion and oedema in the myocardium tissue after chronic stress exposure in rats (29). Chronic stress may enhance sympathetic nerve activity (SNA) and change the function of the sympathetic nervous system.

Enhanced SNA may cause the development of atherosclerosis and endothelial dysfunction (4, 30). Additionally, mental stress can lead coronary artery vasoconstriction, increased heart rate and also high blood pressure. So, myocardial oxygen supply and demand balance is disrupted (6). Besides, heart is a cold sensitive organ and cold stress causes to increase the metabolism rate of it (31). Because of, the enhanced metabolism rate of the heart leads to increase myocardial oxygen requirement, the confusion of oxygen demand balance and the

energy requirements of the myocardium can be compensated for by enlarged blood vessels that we observed in the SG hearts. In our study, we applied cold stress to rats as one part of stress procedure.

Restraint stress can lead to myocardial injury by causing cardiomyocyte damage (32, 33). Another study has shown that social stress leads to cardiomyocyte contractile dysfunction and myocardial pathologies by causing intracellular Ca^{2+} dysregulation and oxidative stress (8).

In a study conducted in recent years, it has been shown that restraint stress causes cardiac injury by leading to release of glucocorticoids. It has been also reported that a preconditioning mild stress applied to the rats before severe stress could alleviate the excess HPA axis response and cardiac damage (34). Glucocorticoids can stimulate cardiomyocytes damage and apoptosis (35). The serum cortisol levels of the CMS exposed rats were higher than the control rats. This increased cortisol levels could cause the damage of the cardiac muscle cells.

Some research has indicated that anger stress brings about an expansion in cross section diameter of left ventricle and intercellular space, decrease of capillary density, myocardial damage and left ventricular dysfunction (36). It is also known that both acute and chronic stress can cause excessive sympathetic stimulation and microvascular dysfunction, the resulting left ventricle dilatation and myocardial damage is occurred (37). In our study, cardiac volumes were found as increased in the stress-exposed rats when compared to the control ones.

We also observed the presence of many adipocytes in perivascular areas. It has been reported that degeneration in cardiomyocytes occurs, and adipose and fibrotic tissue replace these lost cells in arhythmogenic right ventricular cardiomyopathy (38, 39). However, some researchers have reported that existence of the adipose tissue in the myocardium after myocardial infarction was inversely proportional with viable myocardial tissue (40). It has also been specified that the mononuclear cell infiltration was seen in areas close to degenerative and necrotic cardiac muscle cells (38). Similarly, we have also identified infiltrative mononuclear cells around the degenerative myocardial fibres.

Conclusion

As a result, we concluded that the chronic stress caused myocardial injury containing degeneration and oedema leading the cardiac damage. However, the basis of the stress-induced cardiac diseases could not been exactly explained yet.

We believe that further studies would be useful in better understanding of the issues.

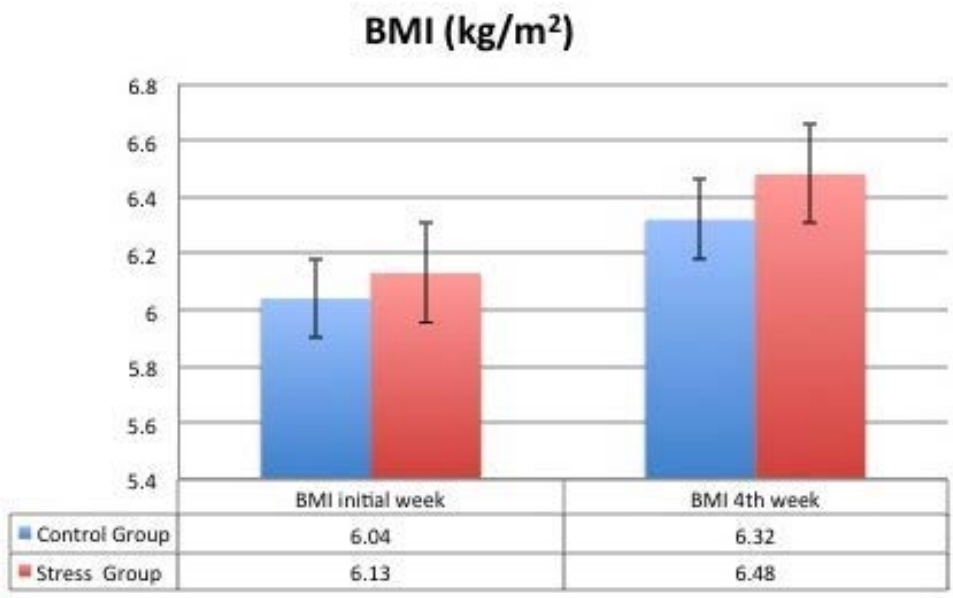

Figure 1. The mean BMIs of the control and stress groups.

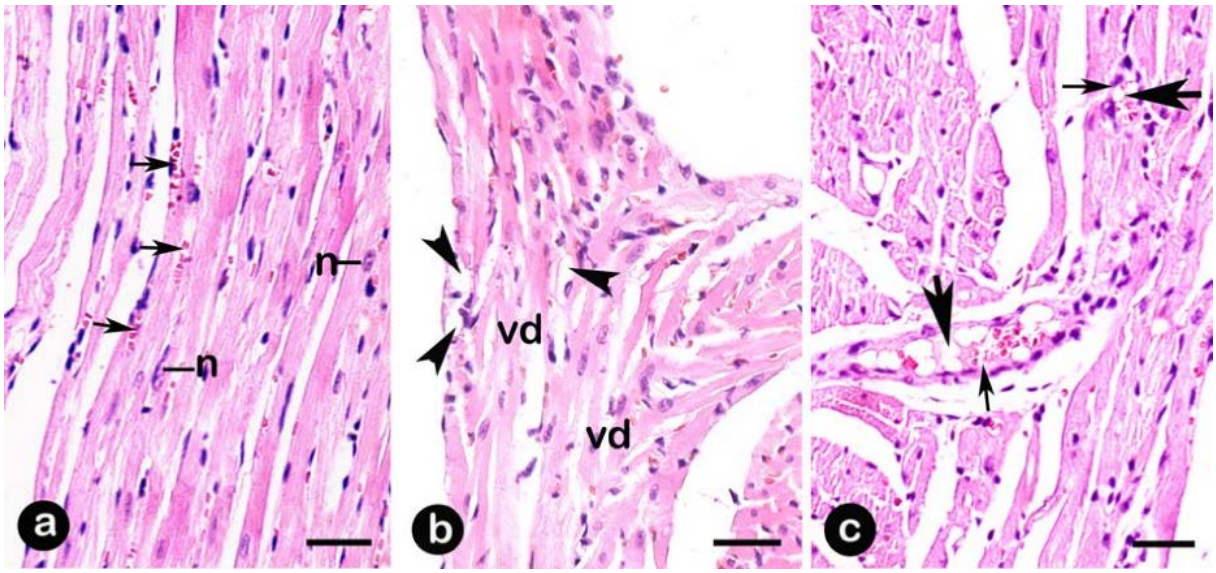

Figure 2. a-c. Light micrographs of cardiac sections of rats. Control group (a). Stress group (b and c). Cytoplasmic swelling, expanded blood vessels and numerous adipocytes within the perivascular stroma are prominent in the stress-performed group. **Arrowhead:** cytoplasmic swelling, **bv:** blood vessel, n: nucleus, **Thick arrow:** adipocyte, **Thin arrow:** blood vessel, **vd:** vacuolar degeneration. **Stain:** H&E. **Magnification bars=** 50 µm

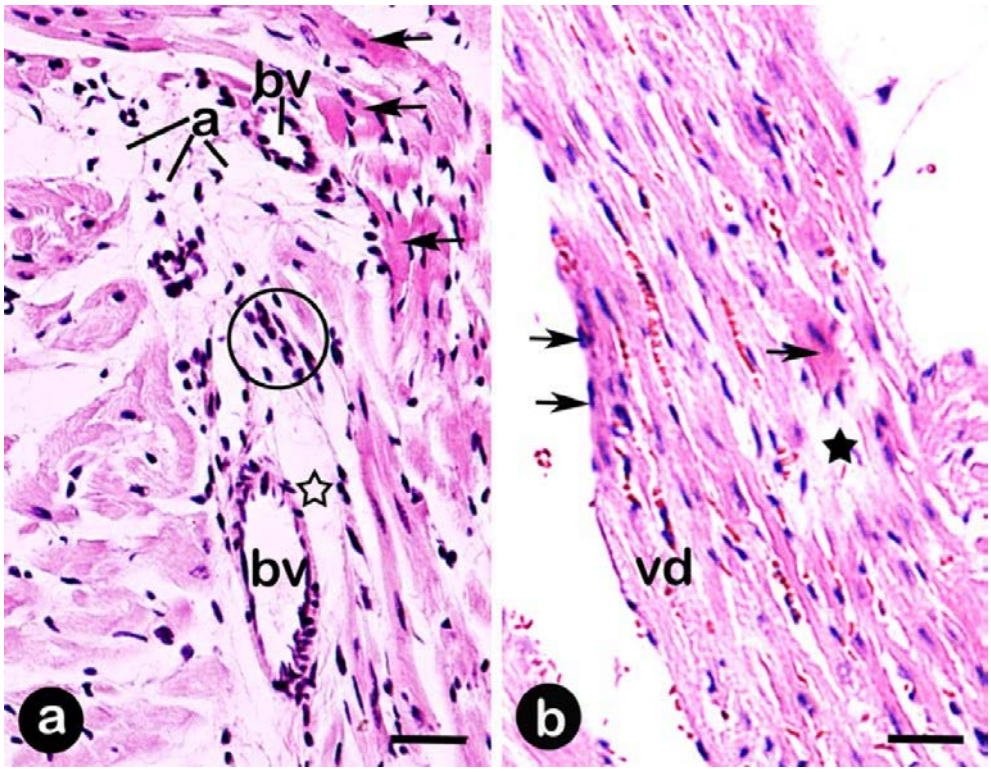

Figure 3. a-b. Light micrographs of cardiac sections of the stress-performed rats. **a:** adipocytes, **Arrow:** necrotic myocardial cells with dense eosinophilic cytoplasm and dark nucleus, **Black star:** interstitial oedema among degenerated myocardial fibres, **bv:** blood vessel, **Circle:** mononuclear cells, **vd:** vacuolar degeneration, **White star:** perivascular oedema. **Stain:** H&E. **Magnification bars=** 50 µm

Groups	**Serum cortisol levels** (µg/dL)	**Heart volumes** (mL)	**p-value**
Control group	1.51±0.26	1.10±0.13	P=0.018
Stress group	2.19±0.26	1. 33±012	P=0.006

Table 1. The serum cortisol levels and heart volumes of the control group and stress group rats

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