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RESEARCH ARTICLE

Are There any Differences in the Expression of Heat Shock Protein 70 in the Liver of the Rats Exposed to Cold Stress in Terms of Gender?

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ABSTRACT

The aim of this study was to reveal whether there is a difference between expression of the liver Heat Shock Protein 70 (HSP 70) and gender in rats exposed to cold stress by using histopathological and immunohistochemical techniques. The study was performed on totally 40 Sprague-Dawley rats [24-week old male (n=20) and female (n=20) rats weighing ~200 and ~220 g, respectively]. The rats were divided into four groups randomly; each group consisted of ten rats (n=10). The first (female) and second (male) groups were accepted as control groups and the third (female) and fourth (male) groups were considered as stress groups. The rats in the third (stress female) and fourth (stress male) groups were exposed to cold (4ºC) 2h/day for 10 days. Then, all of the groups were sacrificed and the livers were collected for histopathological and immunohistochemical evaluations. Histopathological examination of the control group animals showed normal hepatic tissue histology. By contrast, histopathological examination of the stress group animals demonstrated prominent degenerative changes. There were no statistically significant differences in livers of the male and the female stress groups in terms of histopathological changes. The expression of HSP 70 in varying degrees was determined in the liver of both control and stress groups. As severity of lesion increased, the expression of HSP 70 decreased at the same rate. The expression of HSP 70 in livers of the female rats was higher than that of the male rats ($P<0.05$).

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INTRODUCTION

Stress is considered as the psychological and physiological reactions to undesired conditions in the environment, and stimulates the organism. While the organism tries to defend itself against stress, it can also harm itself. The ability to cope with such stressful stimuli is a crucial factor in determining the balance between health and disease (Gibbons, 2012; Rice, 2012). The exposure to cold is one of the important physical agents inducing stress. The exposure to cold as with all stress factors causes oxidative stress and lipid peroxidation in plasma and tissues, leading to damage in the unity of cell membranes (Shore *et al*., 2013; Zhao *et al*., 2014; Wang *et al*., 2015).

Molecular chaperones called Heat Shock Proteins (HSPs) are highly protected proteins. HSPs can protect cells and organisms from different types of damage. They fulfill their functions under normal cellular conditions in every organism. HSPs have various functions such as transporting, folding, unfolding, and gathering the proteins and degraded damaged proteins in the organism (Tutar and Tutar 2010; Tkáčová and Angelovičová, 2012; May *et al*., 2013). Although HSPs are routinely the expression from the cells in a normal levels, at the beginning there the expression increases promptly in response to any changes such as infections (Brenu *et al*., 2013; Terim Kapakin *et al*., 2013b), tumors (See *et al.,* 2011), immunodeficiency (Joly *et al.,* 2010), metabolic diseases (Hooper, 2014) stress (Hasegawa *et al*., 2011; Terim Kapakin *et al*., 2013a), and intoxications (Arawaka *et al*., 2010; May *et al*., 2013) in the organism.

HSPs are generally categorized into different families on the basis of their sequence homology and their molecular masses. The major families contain HSP 100,

HSP 90, HSP 70, HSP 60, HSP 40, and the small HSPs (Tutar and Tutar, 2010; Tkáčová and Angelovičová, 2012).

The HSP 70 is the most protected and the most important constituent of the HSPs when comparing with the others. Also, it is known to be in communication with other HSPs with regard to their functions and release (Tkáčová and Angelovičová, 2012). When cells are exposed to any types of stress, these proteins are expressed at high level in the beginning of the stress. As time goes by, the expression of the HSP 70 is decreased in the prolonged stress situation. Cell damage is observed as a result of this condition (Li *et al*., 2011; May *et al*., 2013; Terim Kapakin *et al.,* 2013a; Terim Kapakin *et al*., 2013b; Pons *et al*., 2013).

So far the investigators have shown that exposure to cold stress have destructive effects on tissues such as adrenal gland (Ferreira *et al.,* 2011), liver (Imamura *et al*., 1997; Kukan *et al.,* 1997; Ateş *et al.,* 2006), heart (Daud *et al.,* 2009; Meneghini *et al.,* 2009), skeletal muscle, and adipose tissues (Davidovic *et al*., 1999).

Recent studies have focused on the expression of HSPs in response to damaged tissues resulted from different causes such as hypertension (Pons *et al.,* 2013), tumors (See *et al.,* 2011), metabolic diseases (Hooper, 2014), heat stress (Terim Kapakin *et al.,* 2013a; Zhao *et al.,* 2014), and intoxication (May *et al.,* 2013).

There are a limited number of studies on relationship between the expression of HSPs in different tissues such as heart and kidney and gender in the rats exposed to cold stress (Voss *et al.,* 2003; Fekete *et al.,* 2006; Shinohara *et al.,* 2013). To the best out of best knowledge, there is no study showing a relationship between expression of HSPs in the liver and gender in the rats exposed to cold stress.

This study was undertaken to reveal whether there is a difference in the expression of HSP 70 in livers of the rats exposed to cold stress in terms of gender by using histopathological and immunohistochemical techniques.

MATERIALS AND METHODS

Animals and experimental design: The study was carried out on totally 40 Sprague-Dawley rats [24-week old male (n=20) and female (n=20) rats weighing ~200 and ~220 g, respectively]. The animals were kept for 7 days prior to the experiment in order to acclimatize. Rats were divided into four groups randomly; each group consisted of ten rats (n=10). The first (female) and second (male) groups were accepted as control groups and the third (female) and fourth (male) groups were considered as stress groups. The rats in the third (stress female) and fourth (stress male) groups were exposed to cold (4ºC) 2h/day for 10 days. Then, all groups were sacrificed and livers were collected for histopathological and immunohistochemical evaluations. This study was approved by the ethics committee of Ataturk University Health Sciences Institute (Decision No: 2009/10/112).

Histopathology: The livers were fixed in 10% buffered formalin and routinely processed for histological examination by embedding in paraffin wax. Tissue sections were cut 4 µm in thickness, and stained by the Hematoxylin-Eosin (HE), the Periodic-Acid Schiff (PAS) and the Masson's trichrome (MTC) for observation under the light microscope (Presnell and Schreibman, 1997).

HSP 70 immunohistochemistry stain: Four µm sections from all the tissue samples were cut and processed for immunohistochemical examination by a standard avidin-biotin-peroxidase method as described by the producer. Mouse monoclonal antibodies that react with human HSP 70 (Clone: BRM-22, Sigma, St. Louis, MO, USA) were used at dilution of 1:500 for 60 minutes. Negative control tissue sections were incubated with normal mouse serum.

Image analysis: Tissue section was evaluated by high power light microscopic examination Olympus Bx52 with DP72 camera system. All immunohistochemically stained section were explored with an image processing system (Olympus, DP2-BSW). For each specimen, HSP 70 and PAS stains were examined in 10 randomly selected areas of approximately X 40 objective. The scores were derived semiquantitatively using light microscopy on the preparations from each rats and were reported as follows: none: − (0), mild + (1): moderate: ++ (2), severe: +++ (3), and very strong: ++++ (4).

Statistical analysis: The data were analyzed statistically using the Kruskal-Wallis Test of nonparametric tests. $P<0.05$ was accepted valuable statistically SPSS (1996).

RESULTS

Macroscopic findings: No differences in macroscopic findings were observed in the livers of the rats in both the male and the female in control groups. The livers in both stress groups appeared to be quite swollen and hemorrhagic.

Microscopic and immunohistochemical findings: No differences in histopathological finding were observed in the livers in both control groups (Fig. 1a). Histopathological examination demonstrated that the hepatic cords were disorganized in rats exposed to cold stress. Remarkable degenerative changes were prominent in the hepatocytes. Also there was necrosis in some hepatocytes. In addition to these changes, inflammatory cells including lymphocytes and histiocytes were present in the perivascular, and periportal regions and parenchyma (Fig. 1b-c). Furthermore, proliferation of Kupffer cells and hyperplasia of bile ducts were observed. Statistically there was no significant difference in the livers of the male and the female rats in the stress groups in terms of histopathological changes.

Reduction of glycogen amount stored in hepatocytes (Fig.1d) was determined with PAS stain. As compared with the control groups, the amount of glycogen in hepatocytes was significantly reduced in both stress groups but there was no statistical significant difference between the female and the male stress groups.

The expression of HSP 70 in varying degrees was determined in the livers of both control (Fig. 2a-b) and stress groups (Fig. 2c-d). As severity of lesion increased, the expression of HSP 70 decreased at the same rate. There was no significant difference in the expression of HSP 70 of the male and the female rats in the control groups. But, in stress groups, HSP 70 in the expression in the female rats was higher than that in the male rats ($P<0.05$).

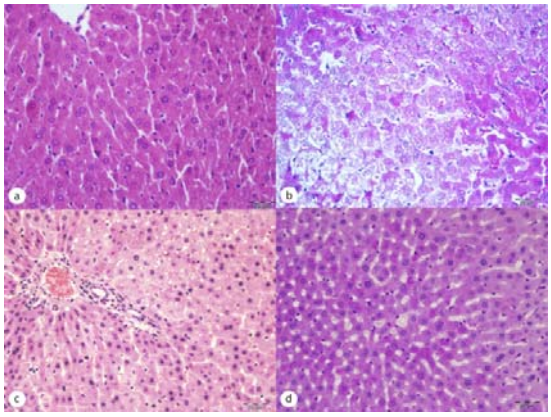

Fig. 1: Photomicrographs of livers of rats showing a) No lesions in control group, *b)* Degeneration and necrosis in hepatocyte, *c)* Inflammatory cells in the perivascular and periportal regions in groups cold stress at male, female , HE, Bar: 20 μm. *d)* Glycogen in hepatocytes PAS, Bar: 20 μm.

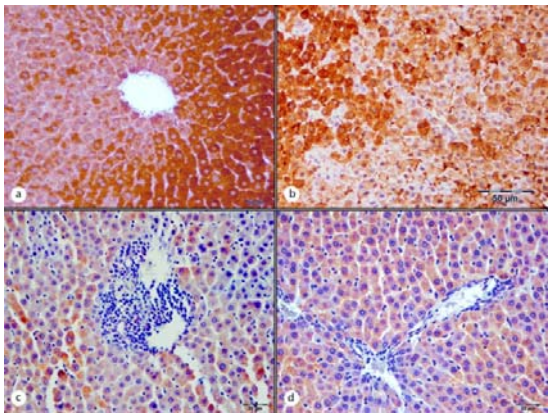

Fig. 2: Immunohistochemstry stain; HSP-70 expression in hepatocytes of liver; a) and b) Moderate in control groups at male, famele, c) and d) Weak in groups stress at male, famele, Bar: 20 μm.

DISCUSSION

Free radicals and complex enzymes are normally produced as a part of defense mechanisms against undesired harmful effects in mammals and humans. Many physiological and pathological conditions, such as infections, emotional changes, and climatic differences, can affect the antioxidant defense system by altering levels of the enzymes and the free radical scavengers (Halliwell, 2012). When the balance between free radical scavengers and antioxidant defense system enzymes is upset, some damage leading to a series of histopathological, biochemical, physiological and behavioral changes occurs in the body. Cold stress is one of the major reasons that disrupt the defense mechanism (Shore *et al*., 2013; Zhao *et al.,* 2014; Wang *et al*., 2015).

Previous studies have reported that the heart tissue (Daud *et al.,* 2009; Meneghini *et al*., 2009), skeletal muscle, adipose tissues (Davidovic *et al.,* 1999), liver (Ateş *et al.,* 2006), venous system (Adan *et al*., 1994), and adrenal gland (Ferreira *et al.,* 2011) can be damaged in the rats exposure to cold stress.

The previous studies demonstrated the presence of dense hemorrhagic areas, degenerative and necrotic alterations in the liver of the rats subjected to cold stress in histopathological examination. Also, hyperplasia in bile ducts and increase in the number of Kupffer cells were observed in these studies (Imamura *et al.,* 1997; Kukan *et al*., 1997). The histopathological changes in the liver observed in the present study were in consistent with earlier ones. There was no statistically significant difference between females and in males in the stress groups (P>0.05).

Stress conditions leads to the release of catecholamines. It increases oxidative glycogenolysis. Hence, oxidative glycogenolysis decreases the concentration of glycogen in the cortical area of the liver cells. (Ferreira *et al*., 2011).

The findings in this study were in consistent with Periodic-Acid Schiff (PAS) special stain. The amount of glycogen in hepatocytes in both the stress groups (females and males) did not show significant (P>0.05) difference.

HSPs are a group of highly protected proteins expressed in most cells under normal physiological conditions in nearly all-living organisms from bacteria to humans. They account for about 5-10% of the total protein ingredient of cells under conditions of normal, healthy growth (Tkáčová and Angelovičová, 2012)

HSPs are expressed under the influence of a wide variety of stresses, including high temperature (Terim Kapakin *et al*., 2013a), immunodeficiency (Joly *et al.,* 2010), infections, (Brenu *et al*., 2013; Terim Kapakin *et al*., 2013b), malignant tumors (See *et al*., 2011) and toxic (Arawaka *et al*., 2010; May *et al*., 2013) and irritant substances (Tkáčová and Angelovičová, 2012). The exposure to cold causes oxidative stress which triggers the production of reactive oxygen species (ROS), suggesting that oxidative stress is a key mechanism mediating HSPs induction (Tkáčová and Angelovičová, 2012). A powerful connection between lipid peroxidation and HSP-70 synthesis in stressed cells is accepted nowadays. These proteins are believed to play important roles in environmental stress tolerance and in thermal adaptation (Li *et al*., 2011; May *et al*., 2013).

Previous studies in rats have shown the relationship between different types stress and the expression of the HSPs (Geiger, 2009; Ishizaka *et al*., 2002; Liu *et al*., 2001; Woerly *et al.,* 2005; Pons *et al.,* 2013; Shahzad *et al.,* 2014). Although HSPs are released in all living organisms at certain rates, under the circumstances like stress, it first increases to protect the cell, then starts to decrease in time and the damage in the tissues occurs (Terim Kapakin *et al*., 2013a; Terim Kapakin *et al.,* 2013b).

In this study, the expiration of HSP 70 was observed in all rats exposed to cold stress. However, the expiration of HSP 70 was less in the areas where the severe tissue damage occurred. The data obtained were in consistent with previous studies.

Recent studies focused on the role of gender in the expression of the HSPs. There have been a few studies regarding the role of gender on HSPs expression in the rats. Voos *et al.* (2003) reported that HSP 72 the expression in the hearts of the female rats was higher than the male rats and level of the HSP 72 in female rats was similar to that in male rats after ovariohysterectomy by using ELISA method. Fekete *et al*. (2006) showed that level of HSP 72 in kidneys of the female rats was higher

than that of the males after renal ischemia. Shinohara *et al*. (2013) indicated that the expression of HSC 70 and HSP 25 were greater in males as compared with females in the control group, but no difference in HSP 70. Also, they stated that gonadectomy had greater effects in males than females, but administration of the exogenous sex hormones induced more vigorous relative changes in females than males. The present study; in stress groups, HSP the expression in female rats was higher than the HSP 70 expression in male rats ($P<0.05$).

In conclusion, this is the first report showing that the gender can be associated with the expiration of HSP 70 in the liver of rats exposed to cold stress by using histopathological and immunohistochemical methods. Our results suggest that the female rats might be better protected against cold stress than the male rats, thanks to higher HSP70 expression.

Author's contribution: KATK, DO and ZK executed and designed the experiments. KATK, SA and ZK performed the necropsy. KATK and SA made histopathologic and immunohistochemical examination. SK performed the statistical analysis. KATK wrote the manuscript. KATK and SK interpreted the data, critically revised the manuscript and approved the final version.

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