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Morphological changes of the hepatobiliary system and salivary glands under the influence of stress

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ABSTRACT

Stress can impact our bodies at the cellular level, causing changes to various organs and systems. Chronic stress can indirectly affect the hepatobiliary tract. Salivary gland dysfunction can be a manifestation of a large variety of systemic diseases or the consequence of local structural or functional pathologies. Stress can influence the oral microbiome, leading to changes in microbial diversity. Scientists study stress reactions, new resistance methods, and modern approaches to prevent and treat stress. It is crucial to manage stress for overall well-being.

The aim is to examine the morphological aspects and changes of the blood vessels in the liver and salivary glands of white rats in experiment using acute immobilization stress.

Materials and methods. In total 22 white male rats were studied, divided into two groups. The first was kept as a control, the second was subjected to acute immobilization stress. The rats were immobilized by hanging them by the neck with a clamp for 6 hours. Animals

were euthanized with a sodium thiopental solution. Samples of the liver and parotid gland were examined histologically using hematoxylin and eosin stain.

Results. The livers of rats were studied after acute immobilization stress. Significant changes were observed in the liver blood vessels, including thrombosis and expansion of perisinusoidal spaces. Liver cells also showed changes such as karyopyknosis, colliquation necrosis, and hydropic dystrophy. The parotid salivary gland histologic examination showed swelling in the connective tissue, periductal regions, and perivascular spaces. In the acinar section, basophilia and swelling of serocytes were observed, along with dystrophic processes leading to remodeling of the acini and ducts. The narrowing of arterioles was observed, reflecting tension in the arterial system.

Conclusions. Exposure to acute stress can cause changes in the liver and biliary tract, increasing the risk of various diseases, including those that affect the oral cavity.

Key words: acini, circulatory bed, hepatobiliary system, liver, oral cavity, salivary gland, serocyte, stress.

Introduction.

Anything that poses a risk to a person's physical and mental health can profoundly impact their overall well-being. Social well-being is also essential to a person's health and happiness. Any threat to this can be quite concerning and should be taken seriously. The issue of stress and its impact on the human body remains relevant [1]. According to the WHO, 45% of all diseases are related to stress; however, some experts believe that this number is twice as high.

The hepatobiliary system and the oral cavity organs, the digestive system's initial part, are functionally and morphologically interconnected. The liver is an endocrine gland, and the salivary glands have both exocrine and endocrine secretions [2]. The hepatobiliary system is one of the most complex multifunctional mechanisms of the human body, which provides the main areas of life: digestion, metabolism (energy supply, metabolism of proteins, fats, carbohydrates, hormones, vitamins, enzymes, water, electrolytes, trace elements, pigments), detoxification, antimicrobial protection, immune responses, as well as blood circulation. A

correlation is established between the condition of the liver and bile ducts and the pathological impact on the oral cavity's organs due to harmful substances, microorganisms, and under-oxidized products. Patients with hepatobiliary tract diseases often experience dental issues, such as periodontitis, atrophic gingivitis, glossitis, hyposalivation, xerostomia, caries, hyperesthesia of enamel and dentin, as well as aphthous and herpetic lesions of the mucous membrane of the cheeks, lips, tongue, and candidiasis [3].

The oral cavity is the first part of the digestive system, in which the initial stages of mechanical and chemical processing of food begin. Saliva plays a vital role in these processes [4]. Saliva constantly washes the mucous membrane of the oral cavity and teeth and is a complex mixture of secretions of numerous small and large salivary glands. The parotid gland produces saliva with a high content of amylase, glycosaminoglycans, and salts. The cells of the parotid gland provide the composition of saliva with antimicrobial substances and the secretion of secretory immunoglobulins into the saliva. The function of the parotid gland depends on the structural state of its secretory part and the bloodstream. According to available scientific research data, dental issues in patients with hepatobiliary tract diseases can be linked to decreased saliva production, dropping of lysozyme, and secretory immunoglobulin A levels. This reduction indirectly activates phagocytes, destroying bacteria, and prevents their colonization of the soft and hard tissues of the oral cavity. As a result, an imbalance of the oral microbiota occurs, leading to oral dysbiosis [5].

Secondary lesions of the salivary glands and other organs of the digestive and hepatobiliary systems occur in many diseases, including stress. The most apparent signs of the development of pathological processes are violations of the morphological and functional state. Violation of liver structure and functions during stress requires great attention and further and thorough study of this effect on the state of the human body as a whole and, in particular, on the state of dental health [6].

The aim of the research is a morphological study of structural changes and violation of blood vessels of white rats' liver and salivary glands in an experimental model of acute immobilization stress by atraumatic hanging of rats by the neck fold for 6 hours.

Materials and methods: The study was conducted on 22 white male rats whose body weight was 200-260 grams, and their ages were 8-10 months. Rats were kept in standard vivarium conditions on a regular diet. The experimental animals were distributed into two groups of 11 individuals each. The first control group comprised intact animals not involved in any experiments. The second experimental group included rats that were exposed to acute immobilization stress.

Fixation by the neck fold was chosen as the basis for reproducing the acute immobilization stress experimental model [7]. For this purpose, non-anesthetized rats were immobilized by hanging by the neck fold with an atraumatic clamp for 6 hours. The experiment was performed from 9:00 AM to 3:00 PM. The animals were removed from the experiment by administering large doses of 10% sodium thiopental solution two hours after the end of fixation.

For histological examination, pieces of the parotid gland and liver were fixed in 10% formalin; after appropriate passage through alcohols of increasing concentration, they were placed in paraffin according to the usual method. The tiny samples were treated with hematoxylin and eosin stain for microscopic examination.

The work was performed by international bioethical principles, in compliance with the provisions of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Results. Stress induces both behavioral and biological responses that activate the hypothalamic–pituitary–adrenal axis, leading to increased levels of cortisol and pro-inflammatory biomarkers that may be involved in developing pathological changes in the hepatobiliary system.

The liver of rats was studied histologically after an episode of acute immobilization stress. The study revealed significant changes in the liver's blood vessels, such as full blood and thrombosis of interlobular and central veins. Additionally, there was a considerable expansion of the perisinusoidal spaces. Most sinusoidal capillaries showed blood stagnation and coagulation, while tissue infiltration by segmented neutrophils, macrophages, and lymphocytes was detected in perivascular and portal tracts. Changes are also observed in liver cells. These include karyopyknosis, subcapsular focal colliquation necrosis in individual hepatocytes, and hydropic dystrophy in hepatocytes located on the periphery of the liver lobes [Figure 1].

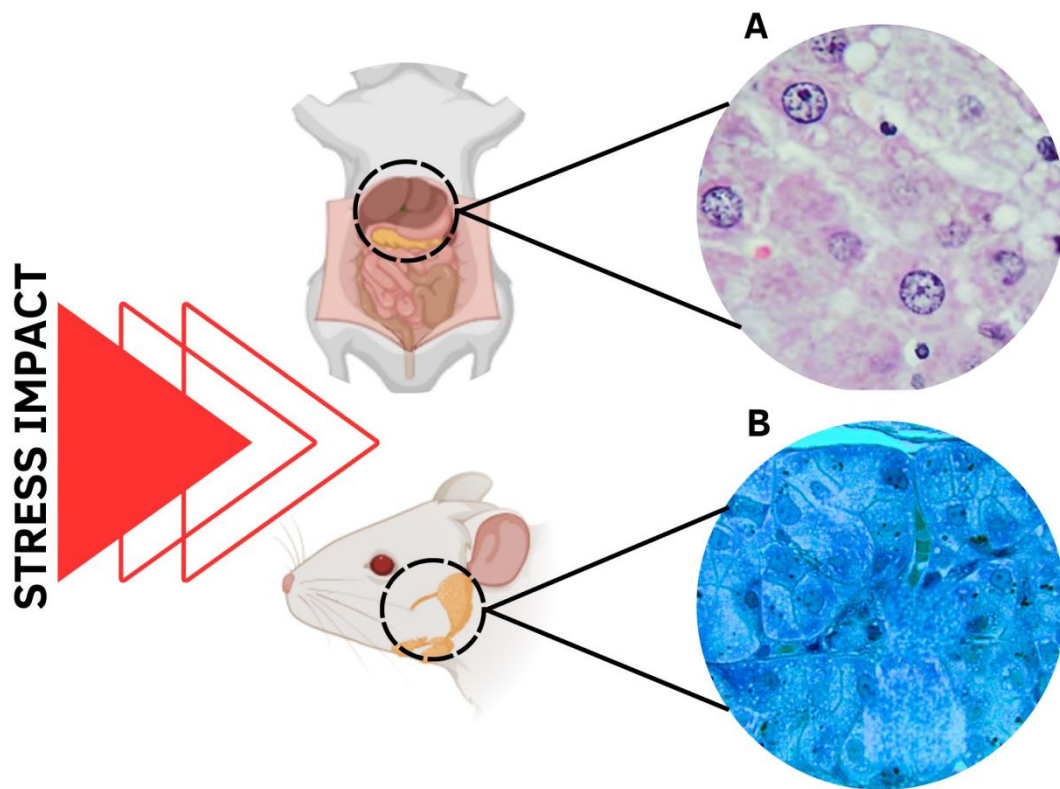


Figure 1. Morphological changes of liver and salivary gland of rats under immobilization stress model

A. Phenomena of karyopyknosis in individual hepatocytes, on the periphery of the lobules, hepatocytes with hydropic dystrophy. Hematoxylin-eosin staining. Magnification x 400

B. Basal arrangement of nuclei in acinar cells of the parotid gland, secretory granules in the apical surface of the serocyte, expanded acinar capillaries. Semi-thin section, stained with toluidine blue. Magnification x 400.

(The figure is created in Biorender.com and Canva.com. platforms Subpictures are original.)

The histological examination of the parotid gland showed swelling of the connective tissue between the acini and lobules. The swelling was also observed in the tissues of the periductal regions and the perivascular spaces. An elevated count of lymphocytes and leukocytes was detected in the stromal areas of the parotid salivary gland.

In the acinar section, there was an observation of basophilia and swelling of serocytes. The glandulocytes' nuclei were mainly located in the basal parts of the cells, and they exhibited an increased optical density [Figure 1]. Intercellular gaps were visible and had an uneven course

due to small expansions along the entire length, sometimes extending to the basement membrane.

Observations have shown the development of dystrophic processes in the cells of the parotid salivary gland. This has led to remodeling of the acini and ducts of the gland, increasing their diameter and area. Additionally, there has been a decrease in the lumen of the excretory ducts and a rise in the number of serocytes, which form the terminal divisions of the acini. These changes have led to a violation of the functional activity of acinar serocytes and epithelial cells of the striated excretory ducts.

Changes in the shape and structure of the parotid gland indicate changes in its blood flow. The arterioles' narrowing was observed, reflecting the general tension of the arterial system. The lumens of the arterioles decreased while the capillaries expanded. Uniform elements of blood were found in many capillaries, which caused the density of the capillary bed to decrease moderately.

Discussion. The ongoing war in Ukraine, the intricate environmental conditions, economic challenges, the faster pace of life, and an overwhelming amount of information are leading to the development of a constantly stressed human body. The number of people experiencing stress reactions is increasing rapidly [8]. The stress reaction is a universal set of protective and damaging mechanisms humans have developed through evolutionary development. This is based on neuroendocrine and metabolic changes due to various internal and external stimuli. Stress has emerged as an essential link in the adaptation process during evolution. However, prolonged or excessive stress responses can become the basis of the pathophysiological mechanisms of various diseases. It is known that the stress reaction is necessary for the body's adaptation to environmental factors. However, as the duration and intensity of stressors increase, the adaptive effect becomes damaging, becoming one of the leading factors in the pathogenesis of most diseases [9,10].

It is important to note that while xerostomia may be related to psychological processes that are not dependent on salivary secretion, depression can decrease the salivary flow rate through anticholinergic mechanisms. This means that psychological conditions can affect both the salivary flow rate and the occurrence of xerostomia. It has been observed that stress can contribute to an increase in salivary cortisol levels. That eventually can lead to changes in the composition of saliva [11]. Changes in the volume or composition of saliva can lead to malfunction in the oral cavity due to its various functions. Research has shown that stress, depression, and anxiety are significantly related to a decrease in unstimulated salivary flow rate and an increase in xerostomia [12].

Scientific research found that animals experiencing acute stress and accompanying cryoinjury show a significant decrease in the speed of bile secretion. It indicates that the liver's biliary function is significantly impaired in the initial stages of injury due to the activation of lipid peroxidation. Lipid peroxidation is one of the major pathogenic factors associated with acute stress [13]. Numerous studies suggest that stress-induced activation of free radical oxidation of lipids can damage the membranes of hepatocytes, leading to hepatitis. In the case of cryodestruction of the skin, previous acute stress can significantly worsen liver damage, even in the late stages of injury [14]. This is likely due to the cumulative effect of systemic pathogenic mechanisms of acute stress and cryoinjury. The traumatic process is typically caused by immune disorders leading to secondary immunodeficiency. Acute stress is known to cause immune suppression, which can decrease the production of antibodies and the phagocytic link of the immune response [15].

In the experiment where rats were exposed to chronic stress, a micro preparation analysis of the liver revealed that there was a significant increase in the number of binucleated forms of hepatocytes, an increase in the relative volume of the stroma, and an increase in the stromal-parenchymal index. Simultaneously, there was a decrease in the relative volume of the parenchyma, indicating severe damage to the liver [16, 17]. Chronic stress can lead to various organic health conditions, such as fibrosis and cirrhosis of the liver, as well as metabolic disorders, such as diabetes and atherosclerosis [18].

Numerous experimental studies revealed the remodeling of oral cavity organs under the influence of hepatobiliary tract diseases [19]. These processes, according to the authors' conclusions, evidenced a dynamic decrease in the functional activity of the parotid gland, which, as a result, could lead to a decrease in the composition of mixed saliva with enzymatic and protein components and were characterized as sclerotic-atrophic with pronounced functional insufficiency [20].

Conclusion. Exposure to acute stress can cause changes in the liver and biliary tract, increasing the risk of various diseases, including those that affect the oral cavity. The leading role in the pathogenesis of salivary gland and hepatobiliary tract diseases under the influence of stress is blood circulation disorders at the level of exchange-type vessels that can lead to organ dysfunction.

Prospects of further research

Our future research aims to explore prevention and correction methods for the adverse effects of stress on the organs of the oral cavity and the hepatobiliary system.

Author contributions

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A – Work concept and design

B – Data collection and analysis

C – Responsibility for statistical analysis

D – Writing the article

E – Critical review

F – Final approval of the article.

Ethical approval

The work was performed by international bioethical principles, in compliance with the provisions of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Ethics Committee of I. Horbachevsky Ternopil National Medical University.

Availability of data and materials

The datasets for this study can be found in the corresponding author upon reasonable request.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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