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LIVER AND HEART DAMAGE IN FEMALE RATS WITH HYPOTHYROIDISM

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Abstract

Background. Hypothyroidism significantly increases the risks of cardiovascular and liver diseases. However, the effect of thyroid hormone deficiency on the functioning of the hepato-cardiac and hepato-renal continuum remain unclear.
The aim of the study was to evaluate the effects of hypothyroidism on the liver parenchyma and myocardium in the experimental conditions.
Study design: Cohort observational study.
Methods. The experiments were carried out on 50 outbred matured female rats with the weight 180-200 g. Animals were randomly assigned to two groups: control (n = 20) and experimental (n = 30).
In rats of the experimental group, hypothyroidism was simulated by daily administration of mercazolil into the stomach using a probe at the rate of 25 mg per 1 kg of body weight for three weeks starting since stage of proestrus. Euthanasia of rats was carried out on the 22nd day of the experiment by total bloodletting from the heart after thiopental sodium anesthesia (60 mg per 1 kg of body weight intraperitoneally). For further experimental research, blood, pieces of the heart and liver were taken. Pieces of internal organs (liver and heart), after fixation in non-sterile formalin and the corresponding processing, were enclosed in paraffin blocks.
The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as the levels of estradiol (ESR), progesterone (PG), thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4), were determined in the blood on a semiautomatic biochemical analyzer "Humalizer-2000" using kits from "Human" (Germany). Additionally, the activity of the enzymes catalase and superoxide dismutase, the content of nitrites, and the content of thyobarbituric acid (TBA) active products: diene (DC) and triene (TC) conjugates as well as Schiff's bases (SB) were assessed.
Statistical processing of digital data was performed using software STATISTICA 14.0 (TIBCO). Differences between groups were calculated using one-way ANOVA with post-hoc Bonferroni correction.
Results. As studies have shown, in the experimental group, the effect of reducing the endocrine function of the thyroid gland. All animals of the experimental group had lower estradiol and progesterone concentration during proestrus and estrus. The development of hypothyroidism in animals was accompanied by the activation of lipid peroxidation manifested by an increase in the concentration TBA active products
Conclusion. 1. In experimental hypothyroidism, oxidative-nitrosamine stress occurs with the involvement of parenchymal organs into the pathological process
2. Pronounced morphological changes in the parenchyma of the liver and myocardium in experimental hypothyroidism indicate the need for timely correction of the thyroid status in a clinical setting.

Keywords: hypothyroidism, experimental model, heart, liver, pathology

Introduction

One of the most common conditions in the practice of an endocrinologist is thyroid hypofunction [1-3]. The leading causes of hypothyroidism are alimentary iodine deficiency and autoimmune thyroiditis (Hashimoto's disease). The incidence of manifested hypothyroidism increases with age, especially in patients receiving amiodarone [1, 2, 4]. According to experts, hypothyroidism affects up to 8% of the population, and another 10-12% of the population may have undiagnosed thyroid insufficiency [1, 3]. Unfortunately, up to one third of patients diagnosed with hypothyroidism does not receive adequate treatment [3].

Hypothyroidism significantly increases the risks of cardiovascular diseases, diseases of the nervous system, infertility, pathology of the musculoskeletal system. In patients suffering from hypothyroidism, the quality of life deteriorates significantly and the risk of premature death increases [1, 4].

Most often, the clinician is faced with primary hypothyroidism. Up to 5% of patients have hypothyroidism for other reasons, including secondary hypothyroidism caused by insufficient production of TSH by the pituitary gland, tertiary hypothyroidism caused by thyrotropin-releasing hormone deficiency, and peripheral (extrathyroid) hypothyroidism [1, 2, 5]. Central hypothyroidism, including both secondary and tertiary hypothyroidism, and peripheral hypothyroidism account for less than 1% of cases [1, 5].

In Ukraine, more than 100,000 patients suffering from hypothyroidism are registered, and the average prevalence of the disease is 250 cases per 100,000 population [6]. For comparison, in Europe, the prevalence of hypothyroidism among the population is 0.2–5.3% [1, 7], and in the United States (NHANES data) - 3.7% [8]. It is assumed that the true prevalence of hypothyroidism in the Ukrainian population may be three times higher [6].

The negative effect of hypofunction of the thyroid gland on the function of the liver and heart has been known for a long time [9-12]. In hypothyroidism, there were revealed an increase in the left ventricular myocardial mass index, a decrease in the ejection fraction, stroke index, thickening of the interventricular septum and the posterior wall of the left ventricle, a decrease in the ratio of the early diastolic flow rate and the flow rate during atrial systole, and an increase in the isometric relaxation phase [9, 10]. Hypothyroid cardiomyopathy is accompanied by the appearance of mucous myocardial edema, deficiency of macroergs, potassium ions in cardiomyocytes, increased lipid peroxidation and membrane damage [9, 10]. This leads to electrical instability of the myocardium, its pseudohypertrophy, accumulation of creatine phosphate, active atherogenesis, impaired rheological properties of blood and microcirculation. Often, especially in elderly patients, hypothyroidism can occur not with bradycardia, but with tachycardia or paroxysms of atrial fibrillation and atrial flutter, polytopic extrasystoles, sick sinus syndrome [10].

Hypothyroidism can have symptoms and indicators similar to those in liver pathology (pseudohepatic symptoms): for example, myalgias, muscle weakness and their twitching, a concomitant increase in the level of aspartate aminotransferase [11, 12]. Sometimes, in severe myxedema, a hyperazotemic coma may occur. Myxedema ascites has been described, in which the ascites fluid usually contains large amounts of protein (exudative form) [12]. It can be a consequence of both liver damage itself with a deficiency of thyroid hormones, and a consequence of right-sided heart failure. This is confirmed by the presence of central congestive liver fibrosis in some patients with hypothyroidism [11, 12]. At the same time, other authors observed normal pressure in the right heart in patients with hypothyroidism, and suggested that increased permeability of the vascular endothelium, leading to the development of ascites, may be a consequence of severe hypothyroidism [1, 4].

There was also evidence that hypothyroidism can directly affect the structure and function of the liver [11]. In some cases, cholestatic jaundice is noted, due to a decrease in the excretion of bilirubin and bile. In experimental hypothyroidism, suppression of the activity of bilirubin-UDP-glucuronyl transferase was detected, leading to a decrease in the excretion of bilirubin. The decrease in the outflow of bile can be partly explained by an increase in the ratio of cholesterol/phospholipids in membranes and a decrease in their fluidity, which may affect the number of tubular membrane transporters and enzymes, including Na^+, K^+-ATPase

[11, 12]. Unfortunately, despite the significant amount of data on the relationship between hypothyroidism and pathology of the heart and liver, the peculiarities of the effects of thyroid hormone deficiency on the functioning of the hepato-cardiac and hepato-renal continuum remain unclear.

The aim of the study was to evaluate the effects of hypothyroidism on the liver parenchyma and myocardium in the experimental conditions

Methods

The experiments were carried out on 50 outbred matured female rats with the weight 180-200 g.

The experiments were carried out in compliance with the norms of the European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes (Strasbourg, 1986), resolutions of the First National Congress on Bioethics (Kiev, 2001) and Order of the Ministry of Health of Ukraine No. 690 [13, 14].

Animals were randomly assigned to two groups: control (n = 20) and experimental (n = 30).

The animals were kept in standard vivarium conditions under natural light, on a standard diet.

In rats of the experimental group, hypothyroidism was simulated by daily administration of mercazolil into the stomach using a probe at the rate of 25 mg per 1 kg of body weight for three weeks starting since stage of proestrus [14]. The stage of estrus circle was determined by the assessment of the cell types observed in the vaginal smear.

Euthanasia of rats was carried out on the 22nd day of the experiment by total bloodletting from the heart after thiopental sodium anesthesia (60 mg per 1 kg of body weight intraperitoneally). For further experimental research, blood, pieces of the heart and liver were taken. Pieces of internal organs (liver and heart), after fixation in non-sterile formalin and the corresponding processing, were enclosed in paraffin blocks.

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as the levels of estradiol (ESR), progesterone (PG), thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4), were determined in the blood on a semiautomatic biochemical analyzer "Humalizer-2000" using kits from "Human" (Germany) [15]. Additionally, the activity of the enzymes catalase and superoxide dismutase, the content of nitrites, and the content of thyobarbituric acid (TBA) active products: diene (DC) and triene (TC) conjugates as well as Schiff's bases (SB) were assessed [15].

The degree of structural disorders of the left ventricle myocardium and liver was assessed by the results of light microscopy [16] with hematoxylin-eosin staining and Schick test.

Statistical processing of digital data was performed using software STATISTICA 14.0 (TIBCO) [17]. Differences between groups were calculated using one-way ANOVA with post-hoc Bonferroni correction.

Results

As studies have shown, in the experimental group, the effect of reducing the endocrine function of the thyroid gland was achieved, as evidenced by an increase in the TSH level to 23.4±1.6 pmol/l (in the control group 14.3±2.2 pmol/l), with a decrease in the level of total T3 to 61.3± 1.9 nmol/l and T4 up to 1.9±0.1 nmol / l (at control levels 75.1±2.8 nmol/l and 3.3±0.3 nmol /l, respectively). All animals of the experimental group had lower ESR and PG concentration in some phases of the estrus cycle. Thus during proestrus the level of ESR in the control group was 88.2±4.4 pg/ml and PG – 38.8±3.2 ng/ml, during estrus ESR - 41.7±3.6 pg/ml, 68.5±4.8 ng/ml, during metestrus ESR – 33.3±3.2 pg/ml, PG – 30.6±3.8 ng/ml and during diestrus ESR – 31.9±2.8 pg/ml, PG – 22.2±2.4 ng/ml. Contrarily, during proestrus level of ESR in the experimental group was 63.4±4.9 pg/ml and PG – 25.5±2.7 ng/ml ($p<0,05$), in estrus ESR – 31.1±4.2 pg/ml and PG – 58.8±4.9 ng/ml ($p<0,05$), in metestrus ESR – 31.6±3.8 pg/ml, PG – 20.4±2.4 ng/ml ($p>0,05$), in diestrus ESR – 32.7±4.2 pg/ml, PG – 19.3±2.2 ng/ml($p>0,05$).

The development of hypothyroidism in animals was accompanied by the activation of lipid peroxidation, which was manifested by an increase in the concentration TBA active products, a decrease in catalase activity against the background of an increase in SOD activity (Table 1). At the same time, the stress of nitrergic systems was noted with an increase in the concentration of nitrite ion. This combination is characteristic of oxidative stress, which plays an important role in the development of autoimmune thyroid diseases.

Simultaneously with the activation of LPO, there was an increase in the activity of transaminases in the main group. Thus, the activity of AsAT increased from 275 ± 58 to 996 ± 38 ue / L, and ALT from 195 ± 46 to 778 ± 32 ue / L. This indicates damage to both hepatocytes and cardiomyocytes caused by oxidative stress.

This assumption is confirmed by the results of a morphological study. In animals of the main group, pronounced dystrophic changes in the liver parenchyma (Fig. 1) and the myocardium of the left ventricle (Fig. 2) were determined

Discussion

On micropreparations of the liver parenchyma against the background of moderate venous hyperemia of the lobules, the lumen of large veins significantly expands, signs of impaired lymphostasis are observed. Hepatocytes are characterized by uneven staining, had signs of granular degeneration. The nuclei had a polymorphic structure. Hemodynamic disturbances in the form of vascular congestion, erythrostasis, erythro- and leukodiapedesis, as well as interstitial edema were observed on myocardial microscope preparations. Dystrophic changes in cardiomyocytes, contracture disorders of myofibrils, focal lymphocytic infiltrates, areas of plasmolysis and fiber fragmentation are noted. Mucous edema with the formation of microcavities was observed in the myocardial stroma.

Both the myocardium and the liver parenchyma are sensitive to a decrease in the local level of thyroid hormones, acting through genomic and non-genomic effects. In this case, the expression of genes of specific receptors for thyroid hormones (TRs) is of great importance [18].

In our previous studies, the relationship between the development of cardiomyopathy and oxidative-nitrosamine stress was shown [19]. Probably, in the case of hypothyroidism, this is the mechanism of alteration. It cannot be ruled out that the correction of the hypothyroid status and the use of antioxidants will make it possible to level the revealed violations.

Conclusions.

1. In experimental hypothyroidism, oxidative-nitrosamine stress occurs with the involvement of parenchymal organs into the pathological process

2. Pronounced morphological changes in the parenchyma of the liver and myocardium in experimental hypothyroidism indicate the need for timely correction of the thyroid status in a clinical setting.

Acknowledgments

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Table 1.

Metabolic profile of the experimental animals

Indices		Control group (n=20)	Experimental group (n=30)
AST, cu/l		275±58*	996±38
ALT, cu/l		195±46*	778±32
NO2-, µM/l		49.8±5.8*	88.8±3.9
SOD, cu/ml		95.3±4.8*	165.5±11.6
Catalase, µM H2O2/ l s		33.3±3.7*	23.6±2.4
TBA active products	DC, c.u.	0.31±0.04*	0.49±0.03
	TC, c.u.	0.25±0.04*	0.10±0.01
	SB, c.u.	1.92±0.12*	9.93±0.86

*. – differences are statistically significant (p<0.05)

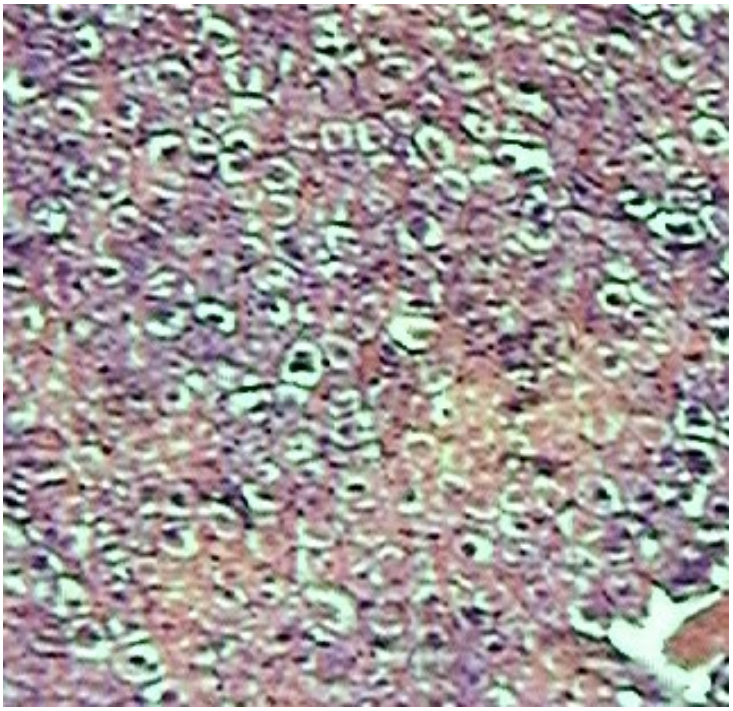

Figure 1: The state of the liver tissue in animals with an experimental model of hypothyroidism. Severe dystrophy of hepatocytes with necrosis of individual groups of cells and plethora. Schick reaction. X100

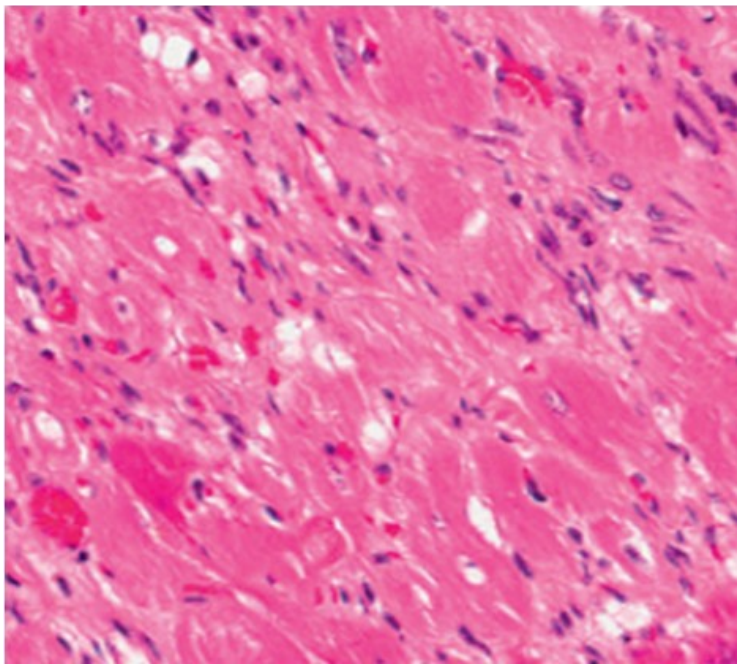

Figure 2 Cardiomyopathy in an experimental model of hypothyroidism. Severe dystrophy of cardiomyocytes. Hematoxylin, eosin. X100.