

Influence of hyperthyroidism on hepatic antioxidants and cytokines levels: An experimental study

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Abstract

Objective: Thyroid diseases greatly affect the liver. Hyperthyroidism can affect the function of the liver. This study aimed to investigate the possible change of antioxidant and pro-inflammatory cytokines levels in liver tissue in hyperthyroid rats.

Material and Methods: This study was carried out with 2 experimental groups. Hyperthyroid group was fed with 4 mg/kg L-thyroxine added standard fodder. Control group was fed with standard rat fodder. Liver selenium (Se) levels were measured by inductively coupled plasma optical emission spectrophotometer (ICP-OES). The antioxidant markers such as Selenoprotein P (SeP), and glutathione peroxidase (GPx), and the pro-inflammatory cytokines such as Interleukin (IL)-18, and Tumor necrosis factor- α (TNF- α) levels were studied in liver tissues by ELISA. All markers levels of liver samples were measured in tissue homogenates.

Results: Se, SeP, and GPx levels of the hyperthyroidism group were lower than the control group. ($p=0.038$, $p=0.046$, $p=0.008$ respectively). There was a significant increase in IL-18 and TNF- α levels in hyperthyroidism group when compared to control group ($p=0.002$, $p=0.023$ respectively). There was positive correlation between FT3 and FT4, IL-18 and TNF- α ($r=0.761$, $r=0.843$, and $r=0.826$ respectively), but there was negative correlation between FT3 and Se, SeP, and GPx ($r=-0.833$, $r=-0.754$, and $r=-0.778$ respectively).

Conclusion: Our findings showed that antioxidant marker levels were decreased, and pro-inflammatory cytokine levels were increased in liver tissues of hyperthyroid rats. These findings suggest that impaired antioxidant and pro-inflammatory status may play a role in liver pathogenesis due to hyperthyroidism.

Keywords: Hyperthyroidism, liver, antioxidant, pro-inflammatory cytokines

Introduction

Hyperthyroidism is a disease characterized by increased levels of thyroid hormones (thyroxine -T4 and/or triiodothyronine-T3), and decreased concentration of thyroid-stimulating hormone (TSH). The primary sites of conversion of T4 to T3 are the liver and kidneys. Thyroid disease is the most common disease that greatly affects the liver. Hyperthyroidism is known to be associated with abnormalities of the liver, including biochemical markers and histology. However, the mechanisms underlying the relationship between hyperthyroidism and hepatic dysfunctions are unclear (1).

Free radicals can oxidize a variety of cellular substances including DNA, proteins, and lipids, leading to changes in cell and tissue functions. Normally the oxidation process is minimized by the antioxidant defense system. However, oxidative stress develops when free radical production exceeds the antioxidant capacity of cells (2).

The high metabolic state in hyperthyroidism is associated with oxygen consumption and the production of reactive oxygen species (ROS), which causes oxidative stress. Oxidative stress decreases the effectiveness of the antioxidant defense system and causes oxidative damage in macromolecules, and tissue damage (3).

Cytokines are small molecular weight proteins that regulate the relationship between tissues and the immune system. These molecules are synthesized by many different cell types and have important roles in health and disease (4). Tumor necrosis factor- α (TNF- α) and interleukin (IL)-1 β are key inflammatory cytokines. They are essentially produced by active kupffer cells, neutrophils, and macrophages. TNF- α , and IL-1 β production may lead to increased defense responses in parenchymal cells by activation of apoptosis. However, the crushing of these defensive responses may lead to necrosis death of the cells, and thus stimulate more inflammatory responses (1).



It has been reported that thyroid hormone disorders increase inflammatory cytokine and ROS production (5, 6). Also, it is studied the liver functions in thyroid diseases. However, it was mostly taken into consideration circulating levels of liver function markers, when examined liver functions in hyperthyroidism (7, 8). Therefore, in our study, we assessed the antioxidant markers such as selenium (Se), Selenoprotein P (SeP), and glutathione peroxidase (GPx), and the pro-inflammatory cytokines such as TNF- α , and IL-18 in liver tissues of hyperthyroidism induced rats. Thus, it is aimed to evaluate the possible effects of these analyzed markers on liver function change due to hyperthyroidism.

Material and Methods

Study design: The experimental study was carried out with 16 Wistar-Albino male rats. Their body weight ranged from 200-250 g. All animals were kept under the same environmental conditions, i.e. at a room temperature of 25°C, with an artificial light cycle (lights: 08:00–20:00 h), and were left for one week for adaptation. The rats were divided into two groups. The first group consisted of controls receiving standard rat fodder. The second group was fed with 4 mg/kg L-thyroxine added standard fodder during 30 days (9). All animals drank tap water. At the end of the 30-day experimental period, the blood samples were collected via cardiac puncture, and liver samples were taken. Serum samples were removed from blood samples after centrifugation at 3000xg for 20 min at 4°C. These samples were stored in polyethylene Eppendorf tubes at -80 °C until analysis.

Hormone Measurements: Serum thyroid stimulating hormone (TSH), free T4 (FT4), and free T3 (FT3) levels were measured by ELISA kits (Sunred biological technology, Shangai, China). All the procedures were carried out following the manufacturer's instructions.

Se Measurement in Liver Samples

Se levels were measured by inductively coupled plasma optical emission spectrophotometer (ICP-OES, Thermo iCAP 6000, Cambridge, UK). The tissue samples were weighed and transferred into metal-free glass tubes for digestion. The samples were first digested with 2 ml of concentrated nitric acid (HNO₃) at 100 °C in the furnace (Merck, Darmstadt, Germany), and 1 ml of perchloric acid (HClO₄) was added to the cooled materials. The materials were then completely digested at 120 °C until the materials diminished to half of the original total volume. Digested materials were diluted with deionized water to 10 ml. Calibration standards were prepared using stock solution at a concentration of 1000 mg/L (Chem-Lab, Belgium). Elemental solutions of 0.0010, 0.0025, 0.0050, 0.0100, 0.0250 and 0.0500 ppm concentrations were prepared by using stock solution and distilled water (Millipore, Bedford, MA, ABD) containing 0.3% HNO₃. Se element levels were determined by using 196.026 nm wavelength. Results were calculated as $\mu\text{g/g}$ wet weight ($\mu\text{g/g}$ tissue).

Preparation of Tissue Homogenates

Liver tissues were excised from all rats, rinsed with ice-cold saline and homogenized in 100 mMTris-HCl buffer (pH 7.4) using a homogenizer. The lysate was then cold-

centrifuged at 12 000 x g for 30 min at 4 °C. Supernatants were collected and stored at -80°C until analyzed. Protein concentrations of supernatants were determined by Lowry's method (10).

Measurement of Antioxidant and Cytokine Levels in Liver Samples

The levels of SeP, GPx, TNF- α , and IL-18 in liver tissue samples were quantified according to the manufacturer's instructions and guidelines using the ELISA kits specific for the rat (Sunred biological technology, Shangai, China). Antioxidant and cytokine levels were determined in the supernatants of tissue homogenates.

Statistical analysis

Data are presented as mean \pm the standard deviation (SD). Statistical analysis was performed using the Student t-test and Mann-Whitney U-test. $p < 0.05$ was considered to indicate a statistically significant difference. In addition, the relationship between variables was investigated by the Pearson correlation test. All calculations were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, Inc., La Jolla, CA, USA).

Results

The Values of Thyroid Function Markers

Comparison of serum FT3 and FT4 levels of experimental groups showed that these parameters were higher in hyperthyroidism group than in control group ($p=0.030$, $p=0.011$, respectively) (Figure 1 A-B). Also, TSH values of hyperthyroidism group were lower than control ($p=0.031$) (Figure 1C).

Antioxidant and Cytokine Values of Liver Tissues

All antioxidant markers of liver samples were measured in tissue homogenates. Liver Se, SeP, and GPx levels of the hyperthyroidism group were lower than control group ($p=0.038$, $p=0.046$, $p=0.008$ respectively). (Table 1).

Also, cytokine markers of liver samples were measured in tissue homogenates. When experimental and control groups were compared for cytokine levels, it was seen that there was a significant increase in IL-18 and TNF- α levels in hyperthyroidism group when compared to control group ($p=0.002$, $p=0.023$ respectively) (Table 1).

Correlation analysis results of all studied markers in hyperthyroid group

When correlation of thyroid functions, antioxidants and pro-oxidant markers in hyperthyroid group were analyzed it was seen that there was positive correlation between FT3 and FT4, IL-18 and TNF- α ($r=0.761$, $r=0.843$, and $r=0.826$ respectively), but there was negative correlation between FT3 and Se, SeP, and GPx ($r=-0.833$, $r=-0.754$, and $r=-0.778$ respectively) (Table 2). Also, significant positive correlation was between Se and SeP, and IL-18 and TNF- α ($r=0.922$, and $r=0.939$ respectively), and negative correlation between FT4 and GPx values ($r=-0.886$) (Table 2).

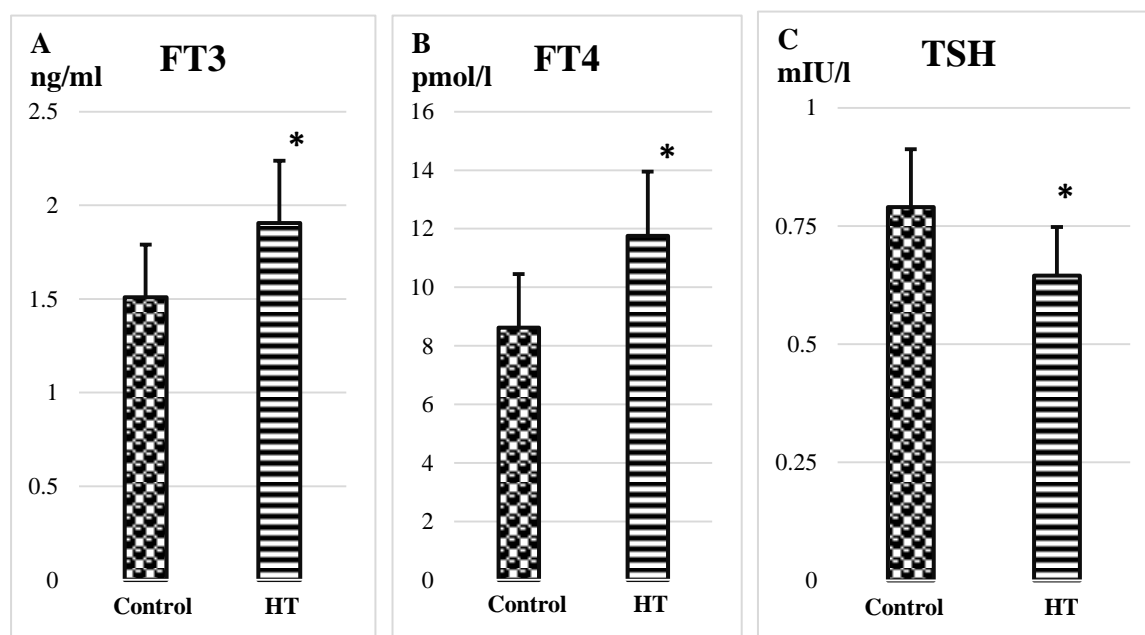


Figure 1A. Serum FT3 levels of studied groups. Values are presented as the mean \pm the standard deviation. * $p=0.030$. **1B.** Serum FT4 levels of studied groups. Values are presented as the mean \pm the standard deviation. * $p=0.011$. **Figure 1C.** Serum TSH levels of studied groups. Values are presented as the mean \pm the standard deviation. * $p=0.031$

Table 1. Antioxidant marker and pro-inflammatory cytokine levels of studied groups

	Control	HT	p
Se ($\mu\text{g/g}$ wet tissue)	2.07 \pm 0.23	1.68 \pm 0.41	0.038
SelP (ng/mg protein)	14.67 \pm 2.03	12.59 \pm 1.77	0.046
GPx (ng/mg protein)	30.79 \pm 2.24	24.34 \pm 5.48	0.008
IL-18 (pg/mg protein)	8.72 \pm 1.54	12.74 \pm 2.44	0.002
TNF- α (pg/mg protein)	29.88 \pm 2.04	32.76 \pm 2.44	0.023

Values are presented as the mean \pm the standard deviation

Table 2. Correlation of thyroid functions, antioxidant marker and pro-inflammatory cytokine levels in hyperthyroid group

	ft3	ft4	TSH	Se	SelP	GPx	IL-18
ft4	p=0.037 r=0.761						
TSH	p=0.151 r=-0.571	p=0.132 r=-0.595					
Se	p=0.015 r=-0.833	p=0.171 r=-0.547	p=0.058 r=0.714				
SelP	p=0.031 r=-0.754	p=0.059 r=-0.694	p=0.016 r=0.826	p=0.002 r=0.922			
GPx	p=0.025 r=-0.778	p=0.005 r=-0.886	p=0.275 r=0.443	p=0.177 r=0.538	p=0.095 r=0.638		
IL-18	p=0.013 r=0.843	p=0.140 r=0.578	p=0.358 r=-0.361	p=0.096 r=-0.626	p=0.155 r=-0.539	p=0.078 r=-0.648	
TNF-α	p=0.017 r=0.826	p=0.076 r=0.670	p=0.314 r=-0.395	p=0.143 r=-0.562	p=0.139 r=-0.566	p=0.069 r=-0.662	p=0.003 r=0.939

Discussion

Thyroid hormones are key determinants of cellular development, growth and metabolism. These hormones control various metabolic activities related to the anabolism or catabolism of carbohydrates, proteins, and lipids to protect homeostasis (11). Thyroid hormones are considered one of the main endocrine regulators of metabolic activity in cells, including hepatocytes (1). Imbalance of thyroid hormones in the body is associated with many chronic diseases, including diabetes mellitus, cardiovascular disease, and liver-related disorders (11). In the liver, the acceleration of aerobic metabolism by T3 enhances the generation of ROS at the mitochondrial, microsomal, and peroxisomal site (12).

Hyperthyroidism is a condition associated with overproduction and secretion of thyroid hormones. Liver dysfunction has been reported in 37% to 77.9% of patients with hyperthyroidism (13). A well-known negative effect of hyperthyroidism is hepatic toxicity, which is characterized by an increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels (3, 13). However, the possible mechanism between hyperthyroidism and hepatic dysfunction has not been fully explained (13). In previous studies, some explanations have been proposed for liver dysfunction associated with hyperthyroidism. It has been found that oxidative stress and mitochondrial oxygen consumption increased in the liver of rats with hyperthyroidism (14, 15). It has been reported that apoptosis in the liver of rats due to hyperthyroidism occurs by activation of intrinsic and extrinsic pathways. These are two classic activation mechanisms of apoptosis. The intrinsic pathway involves the release of cytochrome c from the mitochondria, the production of ROS, and the loss of mitochondrial transmembrane potential. The extrinsic pathway contains cell surface death receptors such as TNF- α and Fas. Binding of TNF- α and Fas ligand to their respective receptors induces apoptosis via apical caspase-8 (16).

In our literature research, it has been seen that there have been many studies examined the relationship between hyperthyroidism and liver oxidant status. Venditti et al. (17) reported that hyperthyroidism was associated with the hepatic levels of oxidized lipids and proteins, leading to increased free radical production in the liver. Fernandez et al. (18) informed that hyperthyroidism increased the activity of the nitric oxide synthase enzyme, increasing the liver's pro-oxidant activity. Assaei et al. (3) reported that GPx and glutathione reductase activities decreased, and malondialdehyde (MDA) levels increased in the liver after T4 application to hyperthyroid rats. These findings have shown that hyperthyroidism increases the production of active oxygen species, hydroxyl radicals, which easily initiate free radical-mediated lipid peroxidation, and result in increased MDA production. Boisier et al. (20) informed that MDA levels were increased and GSH levels were decreased in the liver of hyperthyroid rats (19). Venditti et al. noticed that hyperthyroidism induced in rats by T3 daily injections for 10 days caused significantly increased MDA levels in the liver. Asayama et al. (21) found no change of MDA in liver homogenates from hyperthyroidism induced

rats rendered hyperthyroid by administration of T4 to their drinking water over a 4-week period. But, they also found a low GPx concentration in the liver tissue. Thyrotoxicosis causes liver damage. Malik and Hodgson (22) were reported that the damage may occur due to hypoxia in the perivenular regions due to increased hepatic oxygen requirement.

Se is an important component of selenoproteins that play a role in many biological functions such as antioxidant defense, the formation of thyroid hormones, DNA synthesis, fertility and reproduction (23). SelP, which constitutes more than 50% of plasma Se reserves, is an extracellular glycoprotein. All tissues express SelP, but the liver is the primary source of SelP in plasma. SelP plays a role in carrying Se to tissues and is an important extracellular antioxidant. It eliminates peroxyinitris caused by the reaction of superoxide ions, which are produced in inflammation sites, with nitric oxide (23, 24). GPx is an important antioxidant enzyme. Its main function is to neutralize hydrogen peroxide (H₂O₂) and organic hydroperoxides in the intracellular and extracellular compartments (23).

In this study, we were examined the liver levels of Se, SelP, and GPx, which are known to have antioxidant roles, and were evaluated the effect of these antioxidant markers on the liver dysfunction of hyperthyroidism. Our findings showed that all of these antioxidant markers decreased in the hyperthyroid group. These findings may indicate that the liver antioxidant status is decreased due to hyperthyroidism.

Cytokines are small molecular-weighted peptides, which are located between tissues and the immune system. They either limit damage and suppress the activity and production of pro-inflammatory signals, or induce pro-inflammatory inflammation as a result of infection and injury (25). Some cytokines, such as IL-1 and TNF- α , regulate hepatocyte destruction. They stimulate the synthesis of acute-phase proteins in liver cells and mediate hepatocyte regeneration. In addition, TNF- α and IL-6 have been explained to have stimulating effects on hepatocyte growth (26). There is evidence to support the role of cytokines, including IL-1 α , IL-6, and TNF- α in inflammatory liver disease. These cytokines are produced in the liver by kupffer cells and hepatocytes and play a role in hepatic inflammation (27).

IL-18, a member of the interleukin IL-1 family, is a cytokine with pleiotropic effects. IL-18 is produced by kupffer cells, macrophages, B cells and dendritic cells in lipopolysaccharide stimulation. It has been reported that acute liver damage due to IL-18 can occur through the upregulation of the Fas ligand (28).

TNF- α is a pro-inflammatory cytokine produced by activated macrophages and lymphocytes in response to tissue injury and infection. Serum TNF- α is increased in chronic inflammatory liver disease. In these patients, intrahepatic TNF- α levels are likely to be higher due to local TNF- α production in kupffer cells and hepatocytes. In addition, TNF- α has been associated with ROS production in hepatocytes (29). In our literature research, it has been

seen that studies examining the relationship between hyperthyroidism and liver cytokine level are limited. Tapia et al. (12) reported that the expression of TNF- α , IL-1 β and IL-10 mRNA genes in liver tissues increase in hyperthyroid rats. Fernández et al. (6) observed increased TNF- α and IL-10 mRNA expressions in the liver of T3-treated rats.

In our study, when the findings of liver IL-18 and TNF- α related to hyperthyroidism were examined, it was observed that there were increased IL-18 and TNF- α levels in the hyperthyroid group compared to the control group. These findings may indicate that the liver pro-inflammatory cytokines are increased due to hyperthyroidism.

Conclusion

Our findings suggested that antioxidant marker levels were decreased, and pro-inflammatory cytokine levels were increased in liver tissues of hyperthyroid rats. These findings suggest that impaired antioxidant and pro-inflammatory status may play a role in liver pathogenesis due to hypothyroidism. Mechanisms of pathogenesis need to be investigated with further molecular studies.

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NB: Revisions

Conflict of Interest: The authors declare that they have no conflict of interest

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