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Research article

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Effects of vitamin D treatment on the heart tissue and adropin levels in thyrotoxicosis rats

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Ethics Committee Approval
Adiyaman University Animal Experiments Local Ethics Committee, protocol no.2020/062 Experiments were conducted on animals according to the recommended ethical rules (the National Institutes of Health guide for the care and use of Laboratory animals, NIH Publications No 8023, revised 1978) for the care of laboratory animals.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Thyrotoxicosis is a hypermetabolic disease, common in people with iodine deficiency. Cardiac pathologies can be seen in untreated cases. Vitamin D is a supportive therapy for thyrotoxicosis and its deficiency also plays an important role in pathologies including cardiac diseases. Adropin is a peptide hormone regulating the energy homeostasis, and its levels in blood change in cardiac pathologies. Our purpose is to reveal the effects of vitamin D treatment on the heart tissue of rats with thyrotoxicosis and on the adropin levels.

Methods: Our study was designed as 25 days. 28 Sprague-Dawley female rats were divided into 4 groups; Control (3ml of distilled water), Thyrotoxicosis (100µg/day L-thyroxine) Treatment (100µg/day L-thyroxine+200IU /day Vit. D), Vit D (200IU/day Vit. D). Firstly, heart tissues were stained with Masson trichrome method. The preparations were examined under the microscope and evaluated semi-quantitatively. After that, serum adropin levels were measured with ELISA method. Malondialdehyde level of heart tissue was evaluated by spectrophotometry. Heart tissue was evaluated in aspects of fibrosis, congestion, edema and impairment of tissue integrity.

Results: All of the evaluation parameters of the heart tissue were found highly significantly increased in thyrotoxicosis group, in contrast to the control and vitamin D group. Despite a decrease in the treatment group, there was no significant difference in the thyrotoxicosis group ($P<0.001$). Serum adropin levels of all groups were found to be decreased in contrast to the thyrotoxicosis group. Similarly, tissue MDA levels were significantly higher in the thyrotoxicosis group compared to the other groups.

Conclusion: Consequently, heart tissue damage and differences in adropin levels were found in rats with thyrotoxicosis. It was observed that supportive vitamin D treatment helps to regulate these effects.

Keywords: Thyrotoxicosis, Vitamin D, Adropin, L-thyroxine, Heart

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Introduction

Thyrotoxicosis is a hypermetabolic process that results from the release of large amounts of thyroid hormone into the circulatory system. The most common cause of thyrotoxicosis is Graves' disease followed by multinodular goiter or toxic adenoma. All forms of thyroid pathologies are frequently seen in women, besides iodine deficiency is another major parameter [1]. Thyrotoxicosis syndrome also affects the heart tissue. While rhythm problems and coronary diseases are more common in elderly, tachycardia is more defined in young people [2].

Supportive therapy is one of the treatment strategies in thyrotoxicosis. Vitamin D (Vit D) is a steroid molecule regulate bone metabolism besides calcium and phosphorus levels. Additionally, Vit D deficiency is common worldwide and plays an important role in pathologies including cardiac disease [3]. Vit D has strong antiproliferative, prodifferentiative, and immunomodulatory properties, and also increases the expression of glucose-6-phosphate-dehydrogenase which is protective against oxidative stress [4].

Adropin is a peptide hormone expressed in many tissues including heart [5], and regulates energy homeostasis. Recent data suggests that it has protective effect for endothelial cells, impacts angiogenesis, accelerates blood flow, and intensifies capillarities [6]. Yu et all. showed that adropin levels decreased in acute myocardial infarction [7]. In another study, adropin plasma levels are found to be significantly increased than control group according to the severity of heart failure [8]. These findings revealed the association between adropin and the heart, by making it remarkable target for research.

In this study, we aimed to examine the effects of Vitamin D on heart tissue of experimental thyrotoxicosis rat model, to reveal its relationship with adropin.

Materials and methods

Animals

After smear tests were performed, 28 Sprague-Dawley female rats were divided into 4 groups with 7 animals in each group. The animals were maintained on commercial rat diet that contained 5% fat, 21% protein, 55% nitrogen free extract, and 4% fiber (wt/wt) with adequate mineral and vitamin contents. Each had ad libitum access to food and water. They were kept under a photoperiod of 12 hours of light and 12 hours of darkness in controlled temperature conditions of (20-24ºC). No procedure was performed for 7 days to ensure in-group adaptation of rats. After that, the experimental phase was started, and the experimental period was design as 25 days.

Groups

Control: Each rat was given 3ml of distilled water by oral gavage during the experiment period.

Thyrotoxicosis group: Each rat was given 100μg / day L-thyroxine dissolved in 3ml distilled water by oral gavage during the period [9].

Treatment group: Each rat was given 100μg / day L-thyroxine dissolved in 3ml distilled water, followed by 200IU / day Vitamin D, by oral administration via a dropper during the study period.

Vit D group: Vitamin D was administered orally to rats via the dropper for the duration of the study [10].

24 hours after the last application, experimental animals were anesthetized. Then blood was transcardiacly collected from all rats under anesthesia, and tissue samples were stored under suitable conditions for histological and biochemical studies. Blood samples were centrifuged at 1500 rpm for 15 minutes to separate the sera. Serum samples were stored at -80 ° C until the end of the study.

Ethical committee approval

Experiments were conducted on animals according to the recommended ethical rules (the National Institutes of Health guide for the care and use of Laboratory animals, NIH Publications No. 8023, revised 1978) for the care of laboratory animals (Adiyaman University Animal Experiments Local Ethics Committee, protocol no.2020/062).

Histology

Heart tissues fixed with 10% neutral formaldehyde were embedded in paraffin blocks after a routine tissue follow-up procedure. 4-6 μm sections taken from paraffin blocks were stained with Masson trichome staining method. The prepared specimens were examined under Leica DM500 microscope, evaluated semi-quantitatively and photographed under the light microscope [11]

Serum adropin levels

For the analysis of rat serum adropin levels, ELISA method was used. ELISA kits were commercially obtained from Bio assay technology laboratory Co., Ltd, Shanghai, CHINA (Rat Adropin Catalog no: Cat. No E1069Ra YL). All analyses were performed in accordance with the instructions of the manufacturer, and protein expression levels were calculated by Curve Expert 1.4 (Hyams Development).

MDA levels

Heart tissue samples were homogenized for analysis of the Malondialdehyde (MDA) level. A buffer containing 14.5 mmol Tris base, 36.5 mmol Tris-HCl, 161 mmol KCl and 2% Tween 20 was used for tissue homogenization. 5 ml buffer was added to the weighed samples and homogenized at 16000 rpm for 3 minutes using the homogenizer (Ultra Turrax Type T25-B, IKA Labortechnic, Germany). Homogenates were centrifuged (5000 rpm for 5 minutes) and mixed with 1 ml supernatant, 1 ml 10% (w / v) trichloroacetic acid (TCA), 1 ml 0.6% (w / v) thiobarbituric acid (TBA) and 1 ml distilled water. After 120 minutes incubation at 95 ° C, the mixture was cooled to room temperature. 3 ml butanol was added to each tube after that straightened and centrifuged for 5 minutes at 5000 rpm. The butanol phase was removed and read blank against butanol at a wavelength of 532 nm [12]. Results are given in nmol/g.

Statistical analysis

GraphPad® software program was used for statistical analysis. One-way ANOVA test was used for comparison between more than two groups. Tukey test was used for parametric data and Kruskal-Wallis test for nonparametric data. The statistical significance (*P*-value) level was taken as 0.050 in all tests.

Results

Histology

Heart tissue of rats was performed Masson trichrome staining method (Figure 1). After that, tissues were analyzed in aspects of the fibrosis, congestion, edema and impairment of tissue integrity by histoscoring (Figure 2). All groups were analyzed according to thyrotoxicosis group**.** Similar results were obtained for all aspects. A high increase was seen in the thyrotoxicosis group in contrast to the control and Vit D group (P<0.001). Despite a decrease was shown in the treatment group, significant difference was not revealed (P>0.05).

Figure 1: Masson' trichrome staining results of heart tissue. Scala bar, 200 µm. (a) Control Group: and (b) Vitamin D Group: normal histological view of heart tissue. (c) Thyrotoxicosis Group: Effect of Thyrotoxicosis on heart histopathology in rats remarkable increments of fibrosis, (black arrows), congestion (red arrows); edema and disruption of tissue integrity (black stars). (d) Treatment Group: it was observed that Vit D treatment reduced the heart tissue damage.

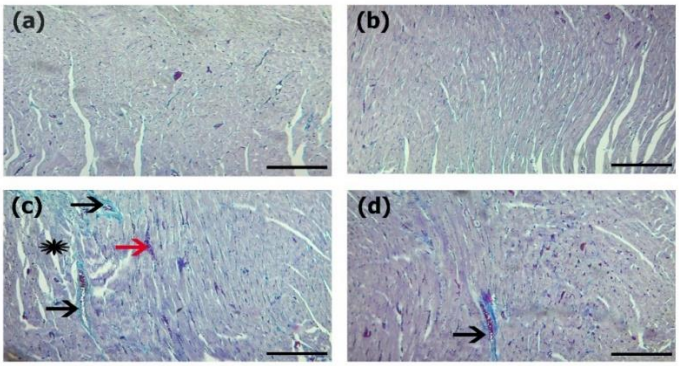
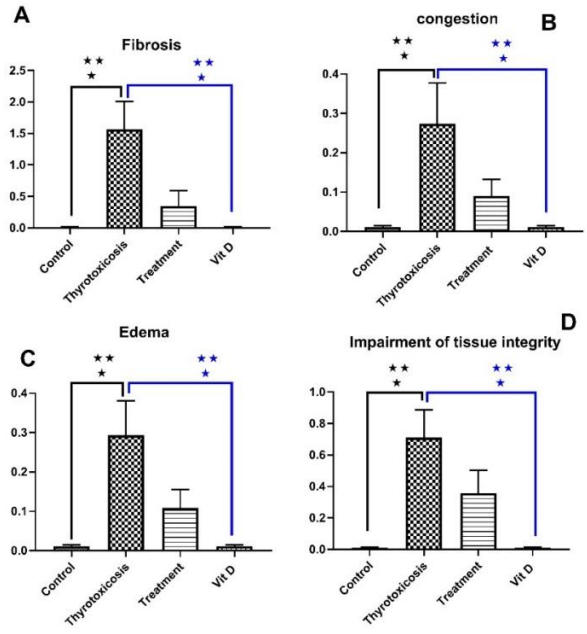


Figure 2: All groups were analyzed according to thyrotoxicosis group. After histoscoring, all groups were evaluated aspects of fibrosis (A), congestion (B), edema (C) and impairment of tissue integrity (D). It was found highly significant increase in thyrotoxicosis group in contrast to the control and Vit D group. Despite a decrease in the treatment group, there was no significant difference.



Serum adropin levels

All groups were compared with the thyrotoxicosis group. Highly significant decrease was found in control group (P=0.005), Vit D group (P<0.001) and treatment group (P<0.001) (Figure 3).

MDA levels

There were seen very high significant decrease in tissue MDA levels in whole groups according to the thyrotoxicosis group (P<0.001) (Figure 4).

Figure 3: All groups were analyzed according to thyrotoxicosis group. Serum adropin levels were found highly significantly reduced in Vit D and treatment group.

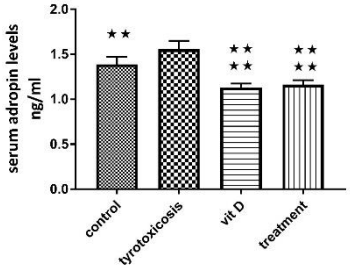
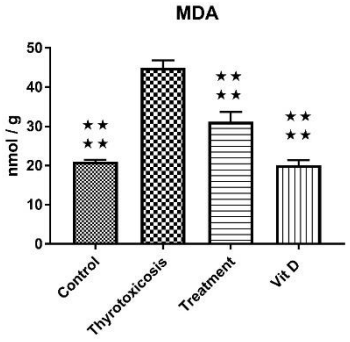


Figure 4: All groups were analyzed according to thyrotoxicosis group. Heart tissue MDA concentrations in all groups were found highly reduced (P<0.001).



Discussion

Thyrotoxicosis is a syndrome characterized by highly increased basal metabolic rate and can cause serious cardiovascular disorders leading to death, if left untreated. The increased metabolic state leads to consumption of oxygen, elevated reactive oxygen radicals, increased metabolic products and acceleration in heartbeat [1]. In addition to the lack of correlation between circulating hormone levels and clinical symptoms, the frequency of symptoms is higher in younger patients [13].

Our results show that patients with thyrotoxicosis have increased fibrosis and impaired tissue integrity in the heart tissue, coherent by the tissue damage effect of thyrotoxicosis. The increased edema and congestion seen in these patients indicate the impairment in the venous return, and thus resulting cardiac problems. All these effects decrease after Vit D treatment, closer levels to the control group. It has been reported that Vit D deficiency is more common in thyroid pathologies [14-16]. These data support that Vit D levels should be measured together with thyroid hormones in thyroid patients, and it makes more important to apply the Vit D supportive treatment.

Lipids in the cell membrane undergo peroxidation by free radicals. The formed products as a result of the reaction join the membrane structure and cause irreversible damage [17]. Measurement of MDA levels, which is an indicator of lipid peroxidation, is one of the methods used to determine the damage. In our study, it was observed that Vit D treatment reduced lipid peroxidation. Another study showed that Vit D support decreased the level of damage in DNA, especially in type 2 diabetes mellitus patients [18]. These results show that Vit D reduces the oxidative damage.

Our findings show that adropin levels increase in patients with thyrotoxicosis, and decrease with Vit D treatment. Previous studies suggested that decreased adropin level is a risk factor for the development of coronary heart disease, but

increased levels are associated with severe heart failure [6]. Kalkan et al. [19] defined that serum adropin and irisin levels were significantly increased in patient with cardiac cachexia. Kumar et al. showed that adropin gene expression decreased in obese mice and administration of adropin reduced the liver steatosis and insulin resistance [20]. Another study emphasized that plasma adropin levels of older rats were lower than younger rats. Besides that, Enho (Energy homeostasis) mRNA and adropin protein levels were decreased in older rat brains [21]. These data suggest that the elevation of adropin in patients with thyrotoxicosis may be the result of increased sympathetic activity and/or tissue damage. From this point of view, adropin levels seem to be more suitable as a prognostic marker rather than treatment.

Limitations

In our study, the number of experimental animals are kept low due to ethical problems. Further studies can be conducted on different antioxidant enzymes and mechanisms that are effective in apoptosis.

Conclusion

In conclusion, tissue damage occurs in the heart tissues of patients with thyrotoxicosis and their adropin levels change. Supportive Vit D treatment has positive effect in both circumstances. We support that Vit D levels should be measured regularly in patients with thyrotoxicosis.

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