

Original Article

Serum apelin levels in patients with thyroid dysfunction

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Abstract: Adipocytes are not only for energy storage, but are also functionally active cells, producing biologically active peptides called adipocytokines. Adipocytokines control nutrition, thermogenesis, immunity, thyroid and reproductive hormones, and neuroendocrine functions. One of the most important new members of this family is apelin. In patients with thyroid dysfunctions, there are usually changes in weight, thermogenesis and adipose tissue lipolysis. Here, we investigated the serum apelin levels in different thyroid hormone states. Our study group consisted of the following patients: 32 thyrotoxicosis, 32 subclinical hyperthyroidism, 31 hypothyroidism, 34 subclinical hypothyroidism and 31 healthy control cases. In addition to routine blood tests, serum free T3 (FT3), free T4 (FT4), TSH and apelin levels were measured, and the body mass index (BMI) was recorded. In terms of the demographic characteristics, age and BMI, there was no statistically significant difference between the groups ($P>0.05$). The mean serum apelin levels of the groups were as follows: thyrotoxicosis group, 4.6 ± 1.9 ng/ml; subclinical hyperthyroidism group, 3.7 ± 1.9 ng/ml; hypothyroid group, 4.8 ± 2.5 ng/ml; subclinical hypothyroidism group, 4.3 ± 2.2 ng/ml; and control group, 3.4 ± 1.4 ng/ml, respectively. There was no statistically significant difference in terms of the mean apelin levels between the groups ($P>0.05$). The hypothyroid group had the highest and the control group had the lowest mean apelin levels. As a result, the apelin levels were higher in both the patients with hypothyroidism and hyperthyroidism, in comparison with the normal population, but without statistical significance.

Keywords: Thyroid dysfunction, apelin, metabolic disorders

Introduction

The role of the thyroid gland is the synthesis and release tetraiodothyronine (T4) and triiodothyronine (T3), which are known as thyroid hormones [1]. Some functions of thyroid hormones are as follows: increasing the basal metabolic rate and oxygen consumption, playing a key role in body temperature homeostasis and energy balance [2]; increasing catecholamine sensitivity by increasing the number of catecholamine receptors in the heart, having positive inotropic and chronotropic effects; inducing osteoporosis by increasing cortisol production and bone turnover; increasing gastrointestinal motility; increasing the contraction and relaxation speed of striated muscle; and controlling the body energy balance and normal growth [3, 4]. Thyroid dysfunctions may be classified as hyperthyroidism, hypothyroidism, subclinical hyperthyroidism and subclinical hypothyroidism, according to the level of thyroid hor-

mones [5]. Hypothyroidism is the second most common endocrine disease, after diabetes. Typical findings of thyrotoxicosis are weight loss, reduction of fat and muscle mass, discharge of fat stores and the decrease in some serum lipids [6, 7]. In hypothyroidism, the oxygen consumption, basal metabolic rate and lipolysis decrease, and the serum triglyceride and cholesterol levels increase. In hypothyroidism, the body weight increases, while thyrotoxicosis is generally associated with weight loss [8].

Adipocytes function not only in energy storage, but are also functional cells. Fat cells produce various biologically active substances with different physiological functions, while adipose tissue expresses the receptors of many of these agents. These molecules have been called adipocytokines or adipokines. Primary adipocytokines are: tumor necrosis factor-alpha (TNF- α), interleukins (IL), plasminogen activator inhibitor

type1 (PAI-1), leptin, apelin, resistin and adiponectin. These molecules have endocrine, autocrine and paracrine effects on different tissues, such as the brain, liver and skeletal muscle. In addition, these molecules control nutrition, thermogenesis, immunity, thyroid and reproductive hormones, and neuroendocrine functions [9]. Clinical and experimental findings in recent years have changed the opinion that adipose tissue only plays a role in the storage and release of energy. Today's opinion is that adipose tissue is a complex and highly active metabolic and endocrine organ [10].

Apelin is an endogenous ligand of the G-protein coupled apelin receptor (APJ), and adipose tissue is the most important source of apelin. In the human body, APJ is expressed by the heart, lung, kidney, liver, adipose tissue, gastrointestinal tract, brain, adrenal glands, endothelium and plasma cells [11].

It is not yet clear if there is a relationship between the blood levels of apelin and thyroid hormones, and if thyroid hormones have an effect on apelin levels, although both apelin and thyroid hormones play important roles in metabolism. From this point of view, it is worth investigating if apelin levels change in thyroid disorders.

Materials and methods

The patients included in this study were evaluated after obtaining ethics committee approval and written informed consent.

Elevated levels of FT3 and FT4, with low TSH levels, were evaluated as thyrotoxicosis. Elevated TSH, and low FT4 and FT3 levels were evaluated as overt hypothyroidism. Subclinical hyperthyroidism was diagnosed with normal FT3 and FT4, and decreased TSH levels. Subclinical hypothyroidism was diagnosed with normal FT3 and FT4, and increased TSH levels [12].

The patients were evaluated in five groups: 1) control group, euthyroid patients (n=31); 2) thyrotoxicosis group, hyperthyroid patients (n=32); 3) subclinical hyperthyroid patients (n=32); 4) hypothyroid patients (n=31); and 5) subclinical hypothyroid patients (n=34). The patients with systemic diseases, such as malignancies, infections or diabetes, and those under anti-

inflammatory and antioxidant therapy were excluded from the study. The BMIs of all patient and control groups were recorded.

The BMI was calculated as the body weight in kg/height² (m²) (kg/m²). After a 12-hour hunger period, venous blood samples were obtained for biochemical analysis, and 5 ml blood samples were centrifuged for 5 min, at 4000 rpm in a mere tube. Portions of the obtained serum samples were transferred to Eppendorf tubes containing aprotinin to analyze the apelin, and stored in a -20°C freezer until the study day.

The thyroid function tests from the blood samples (FT3, FT4 and TSH) were determined using the chemiluminescence method, with an Immulite 2000 analyzer. The serum apelin levels were measured by using the apelin-36 (human) EIA commercial kit (Catalog No. EK-057-15, Phoenix Pharmaceuticals, Inc., Burlingame, USA), with the ELISA method using an ELX 800 ELISA reader. The evaluations were executed spectrophotometrically at a 450 nm wave length.

The data obtained from the study was presented as the mean \pm standard deviation in the text and tables. The SPSS 12.0 for Windows software package program was used for the statistical analysis. Between groups, the differences in the parametric data were analyzed with ANOVA and a post hoc Tukey test, while the differences in the categorical data were analyzed with a Chi Square test. The relationships between the parameters were evaluated by the Pearson correlation analysis method, and the normality of the distribution of the parametric data was evaluated using the Kolmogorov-Smirnov test. P<0.05 was accepted as significant.

Results

In terms of the demographic characteristics, age and BMI, there was no statistically significant difference between the groups (P>0.05). The demographic characteristics of the patients are presented in **Table 1**.

The apelin levels of the groups were as follows: thyrotoxicosis group 4.6 \pm 1.9 ng/ml; subclinical hyperthyroidism group 3.7 \pm 1.9 ng/ml; hypothyroid group 4.8 \pm 2.5 ng/ml; subclinical hypothyroidism group 4.3 \pm 2.2 ng/ml; and control group

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Table 1. Demographic characteristics of the groups

	Control (n:31)	Thyrotoxicosis (n:32)	Subclinical hyperthyroid (n:32)	Hypothyroid (n:31)	Subclinical hypothyroid (n:34)
Age (yil)	46.6±14.8	48.4±18.2	50.3±15.5	46.3±16.9	44.7±13.4
BMI (kg/m ²)	25.5±3.8	24.9±6.8	25.5±4.6	27.6±5.5	26.6±5.3
Apelin (ng/ml)	3.4±1.4	4.6±1.9	3.7±1.9	4.8±2.5	4.3±2.2

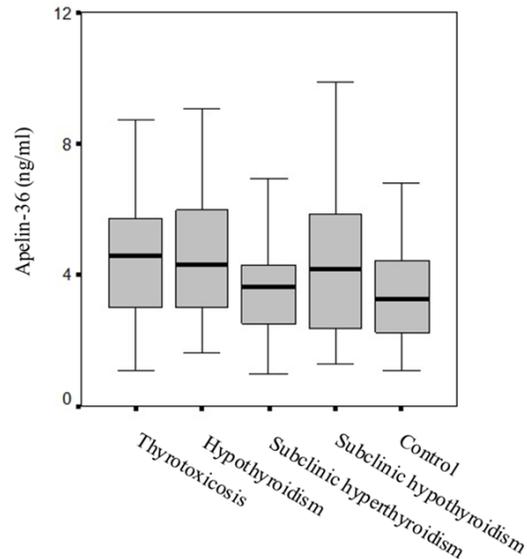


Figure 1. Apelin levels of groups.

3.4±1.4 ng/ml, respectively. The apelin levels were the highest in the hypothyroid group, and then, in thyrotoxicosis, subclinical hypothyroidism, subclinical hyperthyroidism, and the lowest in the control group. There was no statistically significant difference between the groups in terms of apelin ($P>0.05$). The correlation analysis revealed that there was no relationship between the groups ($P>0.05$). However, the apelin levels were always higher in the patients with thyroid dysfunction than in the euthyroid patients (**Figure 1**).

Discussion

Thyroid disorders are very common around the world, and hypothyroidism is the second most common endocrine disease, after diabetes. Thyrotoxicosis occurs less frequently than hypothyroidism, and its prevalence varies between 0.5% and 2%, while both disorders are about 10 times more common in women than in men. The most common cause of hypothyroidism is chronic autoimmune thyroiditis

(Hashimoto's thyroiditis). Hypothyroidism due to radioactive iodine treatment and subtotal/total thyroidectomy are next most common causes. The most common cause of thyrotoxicosis is Graves' disease, which has been reported to be responsible for 60-80% of thyrotoxicosis in different societies. Other diseases that cause thyrotoxicosis are toxic nodular goiter (5-10%) and various cases of thyroiditis [13]. The change in the thyroid hormone levels in both directions may affect metabolic, physiological and biochemical processes, and almost all systems of the body.

In cases of adipocyte dysfunction, such as that seen in obesity and lipodystrophy, a change in the release of adipocytokines and metabolic and energy disorders occur [14]. These adipose tissue dysfunctions affect the energy metabolism by affecting thyroid functions [15]. In patients with thyroid dysfunctions, changes in weight, thermogenesis and lipolysis in the adipose tissue occur. Patients with hypothyroidism usually gain weight, and have a reduction in body temperature and metabolic rate; however, in hyperthyroidism, there is weight loss despite an increased appetite and increased metabolic rate. Most of these metabolic differences are due to the changes in the adipose tissue. Thyroid hormones and adipocytokines are affected by body weight, body fat mass, body temperature, insulin resistance, and glucose and lipid metabolism, and also affect these metabolic events. It is known that there are TSH and thyroid hormone receptors in adipose tissue, and apelin receptors have been detected in the thyroid gland. Therefore, changes in the thyroid hormones and TSH may affect the release of adipocytokines, so there is a possible relationship between thyroid status, thyroid dysfunction and adipocytokines [16, 17].

Mitochondrial inner membrane carriers, which distribute the protein gradient in mitochondria, make use of energy stored in the mitochondria for heat formation. Uncoupling proteins

expressed by the skeletal muscle and brown adipose tissue are thought to play a role in the effects of thyroid hormones on the metabolism. In particular, uncoupling protein 3 expression was increased in rats given T3 [18, 19]. It has been shown that apelin applied to the mice increases the peripheral energy expenditure markers uncoupling protein-1 and uncoupling protein-3, which regulate the lipid export of skeletal muscle. In this way, apelin increases the body temperature and oxygen consumption, and causes weight loss in mice [20].

In rat models of hyperinsulinemia-dependent obesity, the apelin expression of fat tissue and plasma apelin levels increase. Apelin expression is strongly inhibited in fasted rats. However, plasma apelin and insulin levels were found to be significantly elevated in obese patients. It has been shown that there is a strong relationship between insulin and apelin secreted by adipocytes. Additionally, it has been shown in rat models that plasma apelin concentrations and adipocyte apelin mRNA levels increase in hyperinsulinemia associated obesity [21].

In the literature, the only article on apelin levels and thyroid disorders showed that in patients with subclinical hypothyroidism, the apelin levels did not differ from the healthy control subjects [22].

Studies with apelin-like bioactive peptides, such as resistin, adiponectin, leptin and ghrelin, revealed different results. Leptin activates the thyroid gland by affecting specific receptors in the paraventricular hypothalamic nucleus, thus increasing the release of TRH in humans. In humans, leptin receptor mutations are associated with central hypothyroidism [23]. Negative correlations have been found between the TSH and adiponectin levels in obese women, and a positive correlation was found between adiponectin and free thyroid hormones in healthy euthyroid persons [24]. Another study revealed a positive correlation between serum resistin and FT3 and FT4 levels, but a negative correlation with TSH, while the successful treatment of hyperthyroidism was shown to be associated with decreased resistin levels [25]. Ghrelin levels are lower in hyperthyroidism, and antithyroid medication causes ghrelin levels to return to normal levels. Thyroid hormones also have a direct inhibitory effect on ghrelin [26].

These conflicting results are thought to be associated with the etiology of hyperthyroidism, because in patients with autoimmune hyperthyroidism (Graves' disease), the serum adiponectin levels were found to be increased; however, in patients with non-autoimmune thyroid disorders, the serum adiponectin levels did not differ [27, 28].

In our study, the serum apelin levels were the highest in the hypothyroidism group, and then in thyrotoxicosis, subclinical hypothyroidism, subclinical hyperthyroidism, and the lowest in the control group. There was no statistically significant difference between the groups in terms of apelin, and the correlation analysis revealed that there were no relationships between groups. However, the apelin levels were always higher in the patients with thyroid dysfunction than in the euthyroid patients.

In conclusion, the possible relationship between thyroid hormones and apelins is very important, and critical to understanding the etiopathogenesis of metabolic disorders such as obesity and diabetes. Therefore, to obtain stronger results to support our findings, studies containing large patient numbers are needed.

Disclosure of conflict of interest

None.

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