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Original Research Article

Copeptin Levels and Thyroid Hormone Dynamics in Patients with Goiter in Kirkuk City

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Abstract: This study investigates the relationship between copeptin levels, thyroid hormone concentrations, and body mass index (BMI) in patients with goiter. The experiment was conducted from the beginning of April 2023 until the end of September 2023, and samples were collected from Azadi Teaching Hospital in Kirkuk City. Sixty blood samples were collected and divided inti two groups: 40 patients with goiter and 20 healthy individuals. The samples analyzed for copeptin and thyroid hormones (T3, T4, and TSH) using enzyme–linked immunosorbent assay (ELISA) and results revealed significantly higher levels of copeptin, T3, T4, and TSH in goiter patients compared to controls, smoking was found to exacerbate thyroid dysfunction, as smoker tend to have higher levels of copeptin and thyroid hormone compared to non-smokers, and patient with goiter were observed to have a significantly higher BMI, highlighting the complex relationship between thyroid dysfunction, smoking, and metabolic processes. In goiter patient elevated copeptin levels were associated with increased metabolic activity, activation of the hypothalamic- pituitary- adrenal (HPA) axis, and heightened systemic inflammation. The study also examined the role of smoking- induced oxidative stress and inflammation in driving these changes.

Keywords: Goiter, Copeptin, Thyroid Hormones, BMI, Smoking.

1. INTRODUCTION

The thyroid gland is one of the most important endocrine glands in the body, as it produces, stores, and releases hormones through the bloodstream. This gland secretes two important types of hormones: Triiodothyronine (T3) and Tetraiodide (T4), also called Thyroxin. These hormones contain iodine within their composition, and one of their most important functions is regulating metabolic processes in the body [1].

The imbalances that occur in the thyroid gland greatly affect the body, and one of these imbalances is excessive goiter, which causes an enlargement that appears in the front part of the neck area. This disease can be diagnosed through examination or radiological imaging study, and this enlargement is a reaction of the cells. The follicle in the gland suffers from any defect that prevents its production of hormones [2]. The cause of this disease may be attributed to several physiological causes, the most important of which is iodine deficiency, which is part of the synthesis of thyroid hormones. Therefore, this the deficiency will negatively affect its functions and production of hormones, causing an enlargement of the thyroid gland, especially in people who do not consume appropriate amounts of iodine in food [3], in addition to the inflammation that occurs in the thyroid gland may be the cause of its enlargement, such as autoimmune thyroiditis and suppurative thyroiditis, and usually the swelling goes away when the inflammation is treated [4].

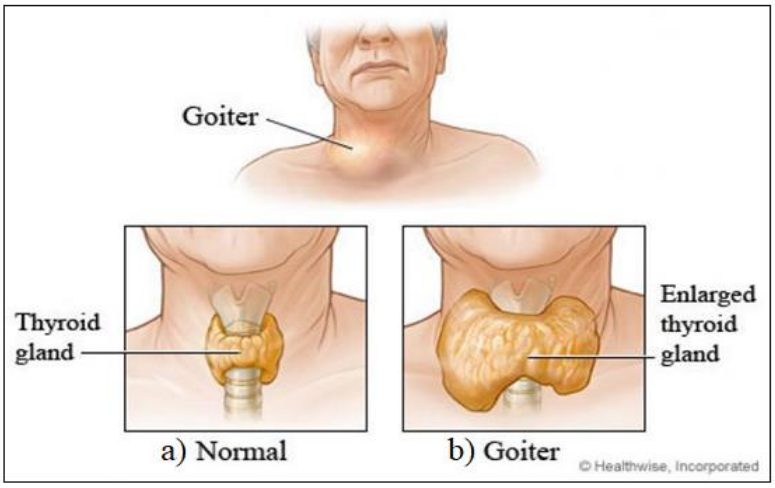

Figure 1: a) Normal Thyroid Gland, b) Goiter

The rate of infection with Goiter depends on the rate of iodine deficiency and statistics in the Middle East indicated the spread of this disease in the Arab countries and the injuries were different if the rate of injury in Egypt was 25.25%, in Algeria 86%, in Bahrain 1.7% [5], and that this deficiency is In iodine leads to a decrease in the production of T3 and T4 hormones, which stimulates the secretion of the TSH hormone from the pituitary gland and there are some stimulating cases to increase the production of TSH such as teenage and pregnancy and thus the thyroid gland will stimulate to produce more hormones, but the deficiency of iodine will hinder this process, which leads to enlarged thyroid gland [6].

To diagnose the causes leading to this disease, there are several indicators. One of the modern indicators is the hormone Copeptin, which is known as the diabetic peptide associated with [7], The two Cubps is one of the sugary peptides and consists of 39 Aminia scandals [8], and the two Cubps are made of the same gene from which the pasoprinin is made in the twentieth chromosome on site 13 and in reverse order with the oxytocin gene and is derived from the third xon from the third coding Xons in MRNA with the last 17 Amino acid for the end of the carboxylic for uorovycin [9].

The current study aims to use the hormone copeptin as a diagnostic indicator for hyperthyroid goiter and as an alternative marker for the hormone vasopressin, as well as to examine the impact of thyroid hormone dynamics on the affected individuals. Additionally, it seeks to understand the influence of smoking, body mass index, and gender on the incidence of this disease.

2. MATERIALS AND METHODS

2.1 Experiment design and collection sample

The study included 60 samples taken from patients visiting Azadi Teaching Hospital in the city of Kirkuk and divided into two groups: 40 samples from patients with goiter and 20 from healthy individuals as a control group, and the experiment was conducted from the beginning of April 2023 to the end of September 2023. 5ml of blood was collected from each patient, then the serum was separated and placed in Eppendorf tubes. The samples were stored at -20°C until the tests were conducted.

2.2 Assessment of Copeptin Hormone

The standards or diluted samples are pipetted into a coated microtiter plate, and a biotinylated detection antibody specific to Human CPP is added. After incubating, excess conjugate and unbound sample or standard are washed away. Avidin conjugated to horseradish peroxidase (HRP) is then added to each well and incubated. Following this, a TMB substrate solution is introduced to each well. The enzyme-substrate reaction is stopped by adding a stop solution, and the resulting color change is measured using a microplate reader. The concentration of Human CPP in the samples is determined by comparing the optical density (OD) of the samples to the standard curve.

2.3 Assessment of Thyroid Hormone

2.3.1 Measurement of T3 and T4 Concentration:

The concentrations of T3 and T4 hormones in serum were measured using competitive ELISA kits provided by Human (Germany), following the manufacturer's instructions. This method relies on a competitive reaction between native antigens in the serum and an antigen-enzyme conjugate for binding to immobilized antibodies on the microplate. Higher antigen concentrations result in less binding of the conjugate, leading to reduced color intensity after the substrate reaction. Absorbance was measured using a microplate reader, and hormone concentrations were calculated based on the standard curve [10].

2.3.2 Measurement of TSH Concentration:

The TSH hormone concentration was measured using the ELISA technique with ready-to-use kits from Human (Germany), following the manufacturer's instructions. The quantitative measurement of TSH is based on a direct sandwich immunoassay. The assay involves wells containing immobilized capture antibodies that bind to TSH in the sample. After adding enzyme- linked detection antibodies (horseradish peroxidase, HRP) that bind to a different epitope on the hormone, hydrogen peroxide is released, which oxidizes the color substrate, tetramethylbenzidine (TMB), turning it from blue to yellow. The concentration of TSH is directly proportional to the color intensity in the sample [11].

3. RESULT AND DISCUSSION

Table 1 demonstrates a significant rise in Copeptin and thyroid hormone levels in individuals with Goiter compared to healthy individuals.

Elevated copeptin levels in individuals with hyperthyroid goiter can be attributed to several interconnected mechanisms [12]. Hyperthyroidism induces a hypermetabolic state, increasing energy expenditure and sympathetic nervous system activity. This physiological stress activates the hypothalamic-pituitary-adrenal (HPA) axis, leading to enhanced secretion of arginine vasopressin (AVP). Copeptin, co-secreted with AVP, serves as a stable surrogate marker for AVP levels, thus reflecting this increased secretion [13].

Thyroid hormones elevate cardiac output and vascular tone, stimulating baroreceptors and osmoreceptors. This stimulation further promotes AVP release, contributing to higher copeptin concentrations [14].

Hyperthyroidism is associated with oxidative stress and systemic inflammation, characterized by elevated cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). These inflammatory mediators can activate the HPA axis, amplifying AVP and copeptin secretion [15].

Furthermore, copeptin influences renal function by altering tubular processes, potentially increasing iodine excretion. In hyperthyroid conditions, the thyroid gland's heightened demand for iodine, coupled with copeptin-induced renal changes, may accelerate iodine turnover [16].

Table 1: Copeptin and Thyroid Hormones Levels in Control and Patient Groups

Parameters	Control Mean ± SD	Patients Mean ± SD	Probability
Copeptin (pg\ml)	32.3 ± 7.4	65.5 ± 22.7	≤0.05
T3 (pg/ml)	1.7 ± 0.8	2.5 ± 0.7	≤0.05
T4 (ng/dl)	0.8 ± 0.3	2.3 ± 0.4	≤0.05
TSH (mIU/ml)	1.4 ± 0.3	5.3 ± 2.3	≤0.05

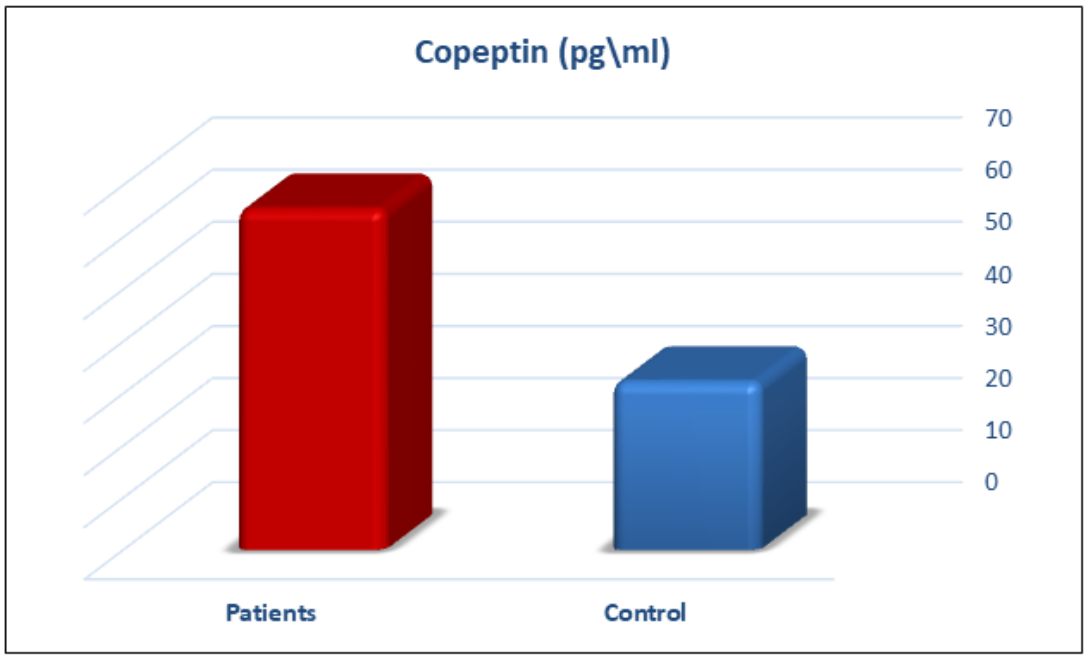

Figure 2: Copeptin levels in patients with goiter and control group

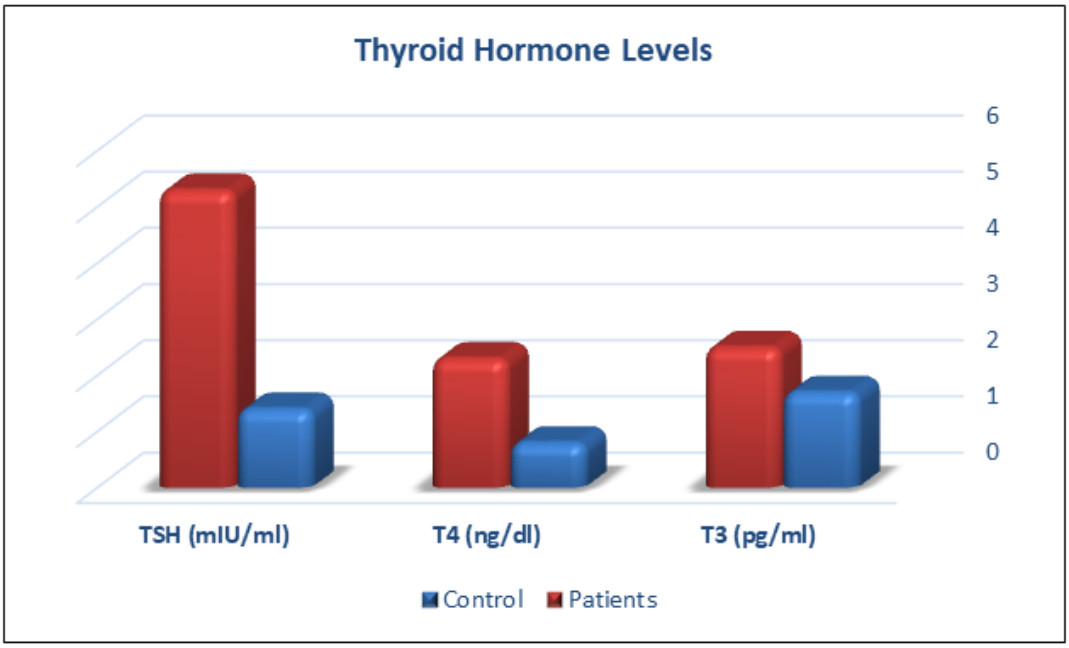

Figure 3: Thyroid Hormone levels in patients with goiter and control group

The results in Table 2 demonstrated significant differences between smokers and non-smokers in Copeptin levels and thyroid hormones.

Table 2: Copeptin and Thyroid Hormones Levels in smokers and Non-smokers Groups

Parameters	**Groups (Smoking)**	**Control Mean ± SD**	**Patients Mean ± SD**	**Probability**	**Probability in patients group ≤**
Copeptin (pg\ml)	smokers	30.4 ± 6.4	62.5 ± 24.3	0.05≤	0.05
	Non-smokers	23.3 ± 6.6	53.1 ± 18.7	0.05≤	
T3 (pg/ml)	smokers	1.6 ± 0.8	2.8 ± 0.6	0.05≤	0.05
	Non-smokers	2.2 ± 0.7	2.3 ± 0.5	0.05≤	
T4 (ng/dl)	smokers	1.3 ± 0.5	2.0 ± 0.4	0.05≤	0.05
	Non-smokers	0.9 ± 0.7	2.1 ± 0.2	0.05≤	
TSH (mIU/ml)	smokers	1.3 ± 0.4	5.3 ± 2.3	0.05≤	0.05
	Non-smokers	1.6 ± 0.7	6.2 ± 2.6	0.05≤	

Smoking is a significant factor influencing thyroid function, often exacerbating the clinical severity of hyperthyroidism, including toxic goiter. The pro-inflammatory and oxidative stress effects of smoking may stimulate vasopressin secretion, indirectly increasing copeptin levels [17]. Moreover, smoking has been associated with increased levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6), which could further enhance AVP release and, subsequently, copeptin levels [18].

In hyperthyroid patients, elevated metabolic activity and vascular changes may synergize with smoking-induced stress, amplifying copeptin levels. This interplay reflects a complex interaction between smoking, thyroid dysfunction, and the hypothalamic-pituitary-adrenal (HPA) axis, leading to an exaggerated stress response [19].

Smoking triggers the production of inflammatory cytokines and boosts oxidative stress, which in turn stimulates the thyroid gland's metabolic activity. This can lead to an increase in the secretion of thyroid hormones such as T3 (triiodothyronine) and T4 (thyroxine), potentially resulting in conditions like toxic goiter or Graves' disease, especially in individuals with a genetic predisposition [20].

Additionally, cigarette smoke contains chemicals like cyanide, which is converted into thiocyanate in the body. Thiocyanate inhibits iodine uptake by the thyroid gland, disrupting the production of thyroid hormones [21].

The Body Mass Index (BMI) was notably higher in the patient group compared to the control group, as shown in Table 3.

Table 3: BMI in the Patient and the Control Group

Parameters	Groups (BMI)	Control Mean ± SD	Patients Mean ± SD	Probability	Probability in patients group ≤
Copeptin (pg\ml)	15-24	30.3 ± 7.4	59.5 ± 22.7	0.05≤	0.05
	25-25	27.2 ± 6.2	66.4 ± 21.3	0.05≤	
T3 (pg/ml)	15-24	1.5 ± 0.8	2.3 ± 0.72	0.05≤	0.05
	25-25	2.3 ± 0.7	2.8 ± 0.6	0.05≤	
T4 (ng/dl)	15-24	0.8 ± 0.6	2.1 ± 0.5	0.05≤	0.05
	25-25	1.6 ± 0.2	3.2 ± 0.6	0.05≤	
TSH (mIU/ml)	15-24	1.7 ± 0.9	6.7 ± 2.3	0.05≤	0.05
	25-25	1.6 ± 0.5	5.3 ± 2.2	0.05≤	

In patients with goiter, the overproduction of thyroid hormones (T3 and T4) usually causes an increase in metabolic rate, leading to weight loss and a decrease in BMI (22). However, some studies have suggested that, in certain cases, particularly in hyperthyroid goiter, BMI may increase due to factors such as fluid retention, increased appetite, or metabolic changes that cannot be fully explained by thyroid hormone levels alone [23].

The results showed an increase in copeptin levels with an increase in BMI, which may be attributed to the stress response caused by thyroid dysfunction. Increased thyroid activity leads to a compensatory increase in cortisol and other stress hormone levels, which in turn results in weight gain and an increase in BMI [24].

The results in Table 4 showed the effect of gender on the studied indicators, as there was a significant increase in copeptin hormone levels in affected males compared to females. Additionally, there was a significant increase in T4 levels in affected males with goiter compared to females.

Table 4: Copeptin and Thyroid Hormones Levels in Male and Female Groups

Parameters	Groups (gender)	Control Mean ± SD	Patients Mean ± SD	Probability	Probability in patients group ≤
Copeptin (pg\ml)	Male	32.3 ± 7.4	65.5 ± 22.7	0.05≤	0.05
	Female	28.3 ± 6.6	57.4 ± 26.7	0.05≤	
T3 (pg/ml)	Male	1.7 ± 0.8	2.6 ± 0.7	0.05≤	0.5
	Female	2.1 ± 0.7	2.8 ± 0.6	0.05≤	
T4 (ng/dl)	Male	0.9 ± 0.5	2.1 ± 0.5	0.05≤	0.05
	Female	1.2 ± 0.7	2.9 ± 0.5	0.05≤	
TSH (mIU/ml)	Male	1.3 ± 0.7	6.3 ± 2	0.05≤	0.1
	Female	1.5 ± 0.9	5.9 ± 2.5	0.05≤	

The reason for the correlation between gender and elevated levels of copeptin may be attributed to several factors, including changes in sex hormone levels in males, such as testosterone, which affects the balance of water and salts in the body, thereby increasing copeptin levels. Additionally, its effect on blood vessels and blood pressure stimulates the secretion of copeptin [25].

3. CONCLUSION

Copeptin hormone levels have been significantly associated with the occurrence of hyperthyroid goiter, making it an important predictive indicator for diagnosing goiter. This association is often due to the effect of copeptin hormone on the hypothalamic-pituitary-adrenal (HPA) axis, leading to increased vasopressin secretion. Additionally, other studied factors such as smoking and high body mass index (BMI) influence the severity of the condition, which is linked to increased oxidative stress and elevated levels of inflammatory cytokines in affected individuals. It is essential to conduct further studies in this field to understand the associated effects and confirm the relationship between copeptin levels and the occurrence of goiter.

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