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Article

Dysregulation of Thyroid Hormone Equilibrium in Hyperthyroidism: A Prospective Case Control Study of T3, T4 and TSH Dynamics

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Abstract: Hyperthyroidism is a neuroendocrine disorder in which the hypothalamic-pituitary-thyroid axis is impaired and metabolism accelerates. Although it is clinically relevant, regional biochemical profiling of thyroid hormone changes has not been well described. To characterize the changes in serum concentrations of triiodothyronine (T3), thyroxin (T4) and thyroid-stimulating hormone (TSH) in hyperthyroid patients and evaluate its distribution according to age and sex. During February 2022 to March 2023, 100 patients (50 hyperthyroid patients (15 men and 35 women) and 50 control subjects exactly matched for age and within the range of 25-65 years) were recruited for a prospective case-control study. T3, T4 and TSH levels were measured by fluorescence immunoassay (iCHROMA™ system). We compared the hyperthyroid patients and the control patients using one-way ANOVA ($P < 0.05$; $P < 0.01$). The hyperthyroid patients had high endocrine dysfunction and T3 and T4 levels were significantly elevated and TSH level was significantly lower than the control patients ($P \leq 0.01$). The highest prevalence was found in the 40-60 year age group thus stratified analysis and age-related risk was also emphasized. There also was strong female presence (64%) and biological sex was also important in the presentation of disease. This significant hormonal disturbance means that an excessive amount of thyroid hormone results in a negative effect on the pituitary by TSH. In this work, we have demonstrated an identified biochemical pattern of hyperthyroidism associated with increased thyroid hormone activity and feedback suppression of TSH. Our results provide regional-based support for demographic determinants of disease distribution and the clinical benefit of combining hormone profiles to detect disease at an early stage and to monitor the disease and keep track of health. These results may also inform diagnostics in some at-risk populations of the disease and may also inform diagnostics in a specific group of patients.

Keywords: Thyroid, Endocrine, Hormones, Pituitary Regulation

Introduction

The thyroid gland is a special endocrine gland located in the anterior neck inferior to the larynx and surrounding the trachea with two lateral lobes connected by a central isthmus [1]. The thyroid gland consists of follicular and parafollicular cells and is responsible for metabolic homeostasis by the production and secretion of thyroid hormones that control multiple physiological processes such as energy metabolism, growth and cell differentiation.

Iodine is needed for making thyroid hormone. It is transported from the blood stream into thyroid follicular cells and used to make hormone. The gland mainly produces thyroxine (T4) and triiodothyronine (T3) hormones, with T4 being the majority of the hormone released into circulation, while T3 is the metabolically active form [2]. T4 is secreted in larger quantities but is converted to T3 , but has much greater metabolic activity at the cellular level [3].

Chronic hyperthyroidism is a clinical syndrome due to the excess production and release of thyroid hormones and hypermetabolic effect on various organs [4]. Dysregulation of the hypothalamic-pituitary-thyroid (HPT) axis results in elevated circulating concentrations of T3 and T4 with suppressed levels of thyroid-stimulating hormone (TSH). This tightly controlled feedback loop is imbalanced and that drives the pathology of the disease. Graves' disease (Diffuse toxic goitre) is the most common cause of hyperthyroidism in the Western world (an autoimmune disease with stimulatory antibodies against the TSH receptor) [5]. [1] Autoantibodies such as thyroid-stimulating immunoglobulins (TSI), thyroid peroxidase (TPO) antibodies and TSH receptor antibodies induce permanent stimulation of the thyroid gland without the normal regulating control of the pituitary gland [6]. Important genetic factors are also involved in Graves' disease and the sex distribution is very uneven with Graves' disease occurring five times more often in females than males. Environmental and lifestyle factors such as stress, smoking, radiation, some medications and viral infections are thought to be the cause of Graves' disease in genetically predisposed people [5]. We have made some progress in understanding thyroid physiology and autoimmune mechanisms, but there is still a need for biochemical characterisation of thyroid hormone changes in hyperthyroid patients in different populations. The objective of this study is to measure serum T3, T4 and TSH levels in hyperthyroid patients and to determine the relationship of age and gender with TSH levels.

Thyroid dysfunction can be caused by many things, including excess or too little iodine, autoimmune diseases, inflammation and cancer. The most common causes of thyroid diseases caused by autoimmunity are immune dysregulation (e.g. hypersecretion as in Graves's disease and hyposecretion as in Hashimoto's thyroiditis). Thyroid pathophysiology: the relationship of genetic susceptibility and environmental causes as shown in this study. In epidemiology there is a huge gender difference in hyperthyroidism with about 5 times as many women as men affected. The current prevalence is approximately 1.3% in the general population and 4–5% for older women. A lot of people's lives are also pretty popular (and less directly associated with risk) phenomenon of smoking. The most common cause of this disease in younger people is Grave's disease and it is toxic nodular goitre for older people [9].

The thyroid gland is a highly vascularised gland located in the anterior part of the neck between the 5th cervical and 1st thoracic vertebrae. The thyroid lies deep to the infrahyoid muscles and consists of two lobes connected by a narrow isthmus. In adults the gland weighs around 15–20 g with sizes which vary with age, sex and physiological status [10], [11]. The specific follicular architecture and its extensive vascularization are important for the highest hormone production and systemic distribution.

Thyroid hormone secretion is regulated by the hypothalamic-pituitary-thyroid (HPT) axis, a tightly regulated negative feedback loop. Thyrotropin (T3) hormone is released from the pituitary gland when the hypothalamus stimulates thyrotropin releasing hormone (TRH) [12]. High levels of T3 and T4 in the circulation inhibit the hypothalamus and pituitary, thereby establishing hormonal homeostasis. Disruption of this regulatory axis leads to overproduction and clinical hyperthyroidism, either as part of autoimmune stimulation, leading to Graves-disease hyperthyroidism, or as part of autonomous production by a thyroid nodule or TSH-secreting pituitary tumour [13].

Thyroid hormone synthesis is one of the most complicated biological processes and depends on iodine and involves, at the molecular scale, iodide uptake, oxidation and organification in thyroid follicular cells. The MIT and DIT which incorporate the iodine into the tyrosine residues of

thyroglobulin, these will later couple to form T3 and T4 [14]. Most of the hormone in circulation is T4. However, T4 is converted to the more biologically active T3 through peripheral deiodination. Most importantly, only unbound (free) levels of these hormones is biologically active. Any change in protein binding, or hormone production can trigger thyrotoxicosis [12].

Some causes of hyperthyroidism include Graves' disease, thyroiditis, excess exogenous thyroid hormone intake and autonomous thyroid nodules. Hormone secreting tumours and iodine induced hyperthyroidism following an iodinated contrast agent used in imaging studies are also frequently responsible [15]. What makes for a complete biochemical and clinical analysis is the heterogeneity of these underlying causes. There are many risk factors that can also cause hyperthyroidism, and they include being female, an older age, family history, and thyroid autoimmune disease. Other risk factors include gestation, dietary iodine fluctuation and viral pathogens [16], [17]. This is a common disease and is associated with ethnicity and diet (especially iodine-rich diets). Clinically, hyperthyroidism is a hypermetabolic state, with weight loss despite increased appetite, intolerance to heat, palpitations, nervousness and fatigue. The patient may also be diagnosed with tachycardia, warm and moist skin, fine hair, lid lag and possibly goitre and gynaecomastia during the physical examination [12], [18].

These symptoms are the result of excess thyroid hormone on various organ systems as a multi-organ systemic effect. Thyroid hormones are necessary for normal growth, development and metabolic homeostasis. Both T3 and T4 are important for skeletal development, energy expenditure and cell differentiation in many tissues [19]. It is well known that deficiency of thyroid hormone causes hypothyroidism and thus developmental defects but excess thyroid hormone causes hyperthyroidism which is characterised by increased metabolic rate and global physiological disorders [20]; [21]. Given the high global clinical burden of hyperthyroidism and the importance of thyroid hormones in physiological homeostasis, characterisation of changes should be done in a clear and thorough manner. Thus, the present study assessed the biochemical profile of T3, T4 and TSH and the association of these parameters with demographic factors in hyperthyroid patients.

Materials and Methods

Out-of-hospital diagnostic laboratories (February 10, 2022 to March 1, 2023). A prospective case-control study of 50 people who were diagnosed with hyperthyroidism and 50 people who were in good health. Participants were aged between 25 and 65. Hyperthyroidism was diagnosed clinically and thyroid hormone levels were measured in the lab by qualified professionals. Patients were divided into patient and control groups to compare them. Symptoms of hyperthyroidism (palpitations, heat intolerance, nervousness, irritability, weight loss, tremors, goiter) were confirmed with biochemical evidence [22]. The control group consisted of healthy age-matched individuals who had no clinical signs of thyroid disorders and no history of endocrine diseases or thyroid medication use, and were recruited from relatives and volunteers among the local community for the study. We excluded patients with known chronic systemic diseases, patients already receiving treatment for thyroid dysfunctions, and conditions that would interfere with thyroid function assessment. All participants underwent a structured clinical evaluation, which consisted of detailed medical history and physical examination. Demographic and clinical information (age, sex, history, and symptoms) were abstracted [23], [24].

Prior to treatment activity, 5 mL of venous blood was drawn under sterile conditions with the consent of every participant. After cleaning the skin with 70% ethanol, blood was collected using sterile 21-gauge syringes. Samples were collected in specific tubes with serum separator gel and centrifuged. Specimens were assigned an individual code for tracking purposes to reduce analytical error [25], [26].

According to the manufacturer (Boditech, South Korea), serum levels of triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) were measured by fluorescence immunoassay (FIA) on the iCHROMA™ system.

T3 and T4 Measurement: The assay was quantitated via competitive immunodetection, where the concentration of analyte is inversely correlated with the fluorescence signal intensity.

TSH Measurement: TSH levels were measured in a sandwich immunodetection assay, using the intensity of fluorescence as a linear indicator of the concentration of TSH present in the sample.

All assays were carried out in a standard laboratory under the same conditions to be analytical accurate and reproducible.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) v.25 was used in data analysis. Results were presented as mean ± SD. One-way analysis of variance (ANOVA) was used to compare between groups. Statistical significance was assigned at $P < 0.05$ and highly significant at $P < 0.01$ (Daniel, 1999).

Results and Discussion

Results

A total of 100 participants were included in this study, comprising 50 patients diagnosed with hyperthyroidism and 50 healthy controls.

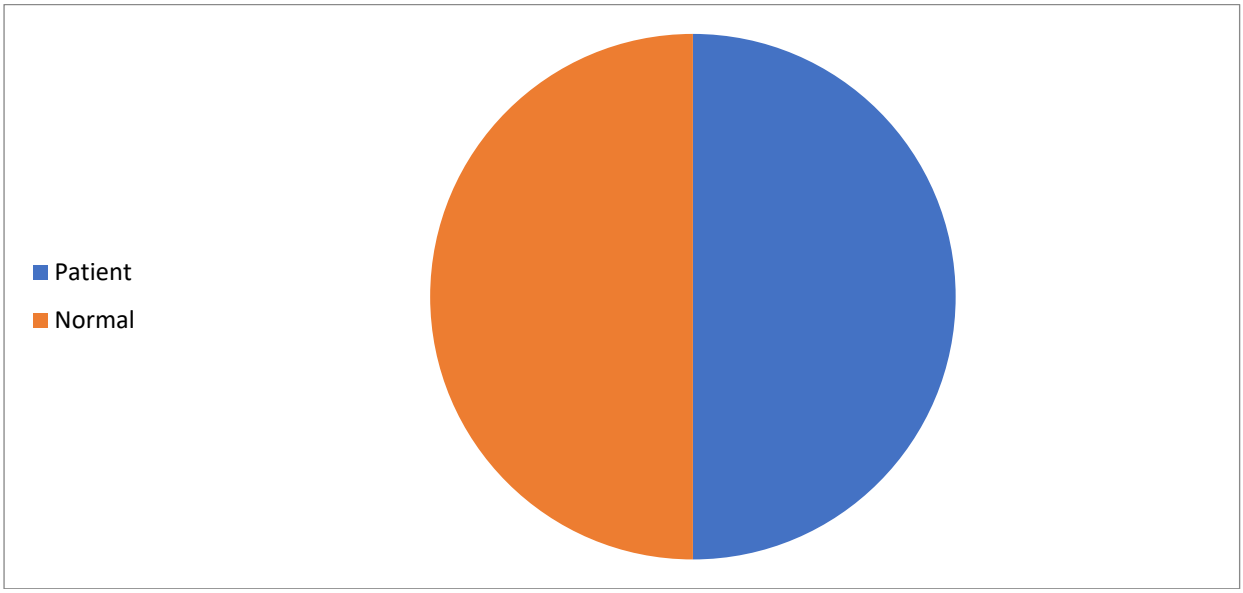


Figure 1. The number of patients group with hyperthyroidism and control group without any signs of hyperthyroidism.

Age Distribution

The distribution of participants according to age is presented in Table 1. The highest proportion of hyperthyroidism cases was observed in the 40–60-year age group (40%), followed by 20–30 years (32%) and 30–40 years (28%). A statistically significant difference was observed between patient and control groups ($P = 0.001$).

Table 1. Age distribution of patients and controls

Age group (years)	Patients (n=50)	Controls (n=50)
20–30	16 (32%)	13 (26%)
30–40	14 (28%)	15 (30%)
40–60	20 (40%)	22 (44%)

P = 0.001 (**highly significant)

Gender Distribution

As shown in Table 2, hyperthyroidism was more prevalent in females (64%) compared to males (36%).

Table 2. Gender distribution in hyperthyroid patients

Gender	Number (n=50)	Percentage (%)
Female	32	64%
Male	18	36%

Biochemical Parameters

Serum Triiodothyronine (T3)

Serum T3 levels were significantly higher in hyperthyroid patients compared to controls (P = 0.001). The mean difference was 0.31 nmol/L (95% CI: 0.09 to 0.53), indicating a moderate effect size (Cohen's d = 0.56).

Table 3. Comparison of T3 level in patients with hyperthyroidism and control group

Parameter	Group	Mean ± SD (nmol/L)	Mean Difference	95% CI	Effect Size (d)	P-value
T3	**Patients**	0.96 ± 0.75	0.31	0.09 to 0.53	0.56 (moderate)	0.001**
	Control	0.65 ± 0.21				

Serum Thyroxine (T4)

T4 levels were markedly elevated in hyperthyroid patients (P = 0.001). The mean difference was 13.8 nmol/L (95% CI: 1.93 to 25.67), with a very large effect size (Cohen's d = 4.57), indicating a strong separation between groups.

Table 4. Comparison of T4 level in patients with hyperthyroidism and control group

Parameter	Group	Mean ± SD (nmol/L)	Mean Difference	95% CI	Effect Size (d)	P-value
T4	**Patients**	18.9 ± 42.76	13.8	1.93 to 25.67	4.57 (very large)	0.001**
	Control	5.1 ± 2.08				

Serum Thyroid-Stimulating Hormone (TSH)

TSH levels were lower in patients compared to controls; however, the difference showed high variability. The mean difference was −2.5 μIU/mL (95% CI: −9.44 to 4.44), with a small effect size (Cohen's d = −0.14).

Table 5. Comparison of TSH level in patients with hyperthyroidism and control group

Parameter	Group	Mean ± SD (μIU/mL)	Mean Difference	95% CI	Effect Size (d)	P-value
TSH	**Patients**	16 ± 18.5	−2.5	−9.44 to 4.44	−0.14 (small)	0.001**
	Control	18.5 ± 16.82				

Discussion

This present study shows the biochemical profile associated with hyperthyroidism, which is elevated T3 and T4 hormones in serum with reduced concentration of thyroid stimulating hormone (TSH) in circulation. The results are consistent with the well known pathophysiology of hyperthyroidism caused by dysfunction of the hypothalamic-pituitary-thyroid (HPT) axis leading to abnormal hormonal feedback [27], [28]. Including effect size analyses helps us understand the size and clinical importance of these differences, not just whether they are statistically significant. The difference in serum T3 levels between groups had a moderate effect size (Cohen's d = 0.56) and resulted in a biologically relevant difference between patients and controls. Even a small increase in T3 levels may play a significant role in hypermetabolic manifestations of hyperthyroidism because T3 is the most biologically active thyroid hormone [3].

In contrast, the serum T4 level is very high (Cohen's d = 4,57) indicating that T4 is highly discriminative to differentiate between hyperthyroid patients and healthy ones. This is in agreement with previous studies reporting significantly high T4 levels in hyperthyroidism [29]. The wide range of T4 may be due to differences in disease duration, severity or physiology and T4 should still be

interpreted in the light of other hormones. TSH also indicated statistically significant but small effect size (Cohen's d = -0,14) and confidence interval that crossed zero, so it was not practically significant. This difference may be due to responsiveness or the stage of pituitary disease and the patient's progress in the disease. The same is true for TSH (also a very sensitive parameter). A single measurement of fT4 or fT2 may vary according to the individual endocrine feedback systems and vascular dynamics [28]. These demographic data are consistent with previous studies. The higher prevalence of hyperthyroidism in women (64%) is consistent with previous studies where the diversity in gender is attributed to the higher prevalence of autoimmune diseases in women and hormonal involvement [30], [31]. Furthermore, the increased incidence in the 40-60 year age group coincides with the age group in which thyroid function and regulation have been shown to change [32], [33].

Finally, the use of effect sizes improves study findings by providing an estimate of the strength of the association (in addition to the P value). The significance of the difference is statistical but effect size also gives an objective view of the magnitude of the difference relative to and this is very important for clinical significance and translational impact in endocrine diseases. Conclusion: Therefore, in this study, the assessment of combined hormones (T3, T4, TSH) is important for diagnosis and assessment of hyperthyroidism. This adds an extra level of sensitivity to the data and provides further evidence of hormonal changes in this disease.

Conclusion

We present detail biochemical and demographic analysis of hyperthyroidism with more significant abnormality of thyroid hormones. However, the hyperthyroid patients had higher levels of triiodothyronine (T3) and thyroxine (T4) compared to the control due to the dysfunction of HPT axis. Effect size analysis showed that T4 was the dominant factor for hyperthyroid patients and controls, followed by T3, and TSH had small to negligible effect size although statistically significant. Our results show that it is important to interpret effect sizes as well as p-values to help make those findings clinical. Demografi, hipertiroidisme paling sering terjadi pada wanita dan orang berusia 40-60 tahun, karena ini sesuai dengan faktor hormon dan usia yang membuat mereka berisiko terkena penyakit. This study emphasises the need for integrated hormonal profile (T3, T4, and TSH) for better diagnostic accuracy and clinical appraisal of hyperthyroidism in the patients. This finding gives sufficiently specific regional evidence of biochemical assessment of endocrine disorder.

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