Original Article

Evaluation of Serum Adipolin Level as a Potential Marker of Metabolic and Hormonal Changes in Patients with Overt and Subclinical Hypothyroidism

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Abstract

Background: Adipokines are a group of cell signaling proteins produced by adipose tissue. These proteins influence appetite, obesity, and insulin sensitivity, and may interact with thyroid hormones. However, the exact relationship between thyroid hormones and adipokines remains unclear. **Objectives:** The aim of this study was to evaluate and compare serum levels of adipolin in healthy individuals and hypothyroidism patients, including subclinical hypothyroidism (SCH) and overt hypothyroidism (OH). **Materials and Methods:** The study's case–control design involved 90 participants, ranging in age from 18 to 55 years Three groups of individuals were created: one group consisted of 30 individuals who had SCH, 30 had OH, and the remaining comprised the healthy control group. Anthropometric characteristics that were assessed and recorded for every individual were age, gender, height, weight, body mass index, and hormonal characteristics. **Results:** People with hypothyroidism had considerably lower serum adipolin levels than those with normal thyroid function ($P < 0.001$). Moreover, there was a negative correlation ($r = 0.667$) between adipolin levels and thyroid-stimulating hormone levels. T4 levels were positively correlated ($r = -0.214$, $P < 0.001$). The receiver operating characteristic curve cutoff area under the curve (AUC) of 0.983, with a value of ≤18.7 in the SCH group, sensitivity of 86.7%, specificity of 100%, and a P value of < 0.001. For the OH group, the cutoff value was ≤14.43, with an AUC of 1.000, sensitivity of 100%, specificity of 100%, and a P value of <0.001. **Conclusion:** The results imply that blood adipolin levels, especially in those with SCH, are an effective biomarker for diagnosing and monitoring hypothyroidism. The study's exclusion criteria confirmed that neither further medication use nor systemic problems impacted the outcomes.

Keywords: Adipolin, hypothyroidism, thyroid-stimulating hormone

Introduction

A condition known as hypothyroidism occurs when the thyroid illness causes the body to produce insufficient thyroid hormone. Primary hypothyroidism is caused by issues with the thyroid gland, whereas secondary or central hypothyroidism is caused by issues with the pituitary gland or hypothalamus. Elevated thyroid-stimulating hormone (TSH) levels in the presence of normal blood levels of free T3 and T4 are indicative of subclinical hypothyroidism (SCH), a frequently occurring type of primary hypothyroidism.[1]

Each year, roughly 2%–5% of patients with SCH progress to overt hypothyroidism (OH). TSH levels above 10 mIU/L in patients who have subclinical or clinically obvious hypothyroidism should seek treatment. Neuropsychiatric symptoms associated with hypothyroidism include depression, memory and cognitive impairments, and impaired motor coordination. Although the average prevalence is 10%, the prevalence increases significantly among older adults, especially among women.[2]

There is ongoing discussion on the causative relationship between hypothyroidism and obesity, despite the consensus that the two illnesses are associated. Although SCH and obesity are not clearly related, a slight increase in weight is linked to OH.[3]

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Hypothyroidism has a wide range of etiologies and presentations. The most typical signs of increasing body mass include hair loss, a change in voice, dry skin, weariness, diarrhea, and susceptibility to cold. However, the appearance and severity of symptoms associated with hypothyroidism may vary depending on age, gender, and other factors. Hypothyroidism, if left untreated, increases the risk of morbidity and mortality.[4] Moreover, hypothyroidism is more common in Type 2 diabetes mellitus patients (DM-II).[5]

Thyroid autoimmune diseases are the most typical cause of hypothyroidism, including Hashimoto's thyroiditis (HT). However, globally, an iodine-deficient diet is the primary cause of hypothyroidism. Today, a simple blood test can be used to detect hypothyroidism, and the only treatment option is to give synthetic thyroid hormone.[6]

The hormonal and immune functions of adipose tissue are primarily mediated by adipocytokines. HT is the most common autoimmune disease affecting thyroid function. Thyroid hormones control metabolism and organ function.[7]

Thyroid hormones have a major influence on fat tissue. Adipose tissue is where fat is mostly stored and is also where lipid transport, synthesis, and mobilization occur.

When a person is hungry or ill, adipose tissue stores metabolic energy as fat for later use. Moreover, adipose tissue functions as a homeostatic mechanism, controlling energy stores and releasing various chemicals to maintain the organism's balance. Some of these substances, such as leptin, function as adjuncts, controlling the amount of fat stored in the body.

In animals, adipose tissue is classified into two types: white adipose tissue (WAT) and brown adipose tissue, each with unique functions, phenotypes, and regulatory systems. Lipid storage has long been linked to WAT. White adipocytes contain large lipid droplets occupying the cellular spaces, with cellular components, such as nuclei and mitochondria, situated close to the cellular membrane. WAT is found in a variety of anatomical locations, including intra-abdominal, visceral, and subcutaneous fat. Because of the link between increasing visceral fat and insulin resistance, metabolic syndrome, and cardiovascular diseases, both types demonstrate unique hormone-dependent lipolytic sensitivity and abundance.[8] In 2011, Takashi Enomoto introduced adipolin (FAM132A/CTRP12 gene) as the newest member of the adipokine family, and it is important in glycemic management and insulin sensitivity. Takashi Enomoto discovered that similar to adiponectin, adipolin administration not only improves insulin sensitivity and glucose tolerance but also results in a reduction in inflammation and adiposity in animal models of obesity and diabetes. In obese mice, adipolin circulation levels dropped significantly. It functions as an anti-inflammatory agent by reducing the expression of pro-inflammatory genes, including tumor necrosis factor-α and interleukin-β, which are important mediators of periodontitis. Moreover, fewer macrophage cells are produced.[9]

The aim of this study was to elucidate the correlation between serum adipolin levels and TSH concentrations in patients with hypothyroidism.

Materials and Methods

Patients and study design

This case–control study was conducted at the Al-Hakim Teaching Hospital in Najaf, Iraq from September 2023 to April 2024. In addition to anthropometric information, such as age, sex, weight, height, and body mass index (BMI), hormonal markers were assessed and documented.

A total of ninety individuals, ranging in age from 18 to 55, took part in the study. These were divided into three groups: 30 participants with SCH patients (13 males, 17 females), 30 with OH (13 males, 17 females), and 30 with healthy individuals (17 females, 13 males) who served as the control group.

Patients were diagnosed with SCH if their TSH level was higher than 4.5 μIU/mL and their T4 and T3 levels were normal, whereas OH was identified in patients with low T4 and T3 levels and increased TSH>10 μIU/mL. Individuals who were on any medication that could affect lipid metabolism, had thyroidectomies, anemia, cancer, or any other obvious systemic disease sickness were excluded from participating in the study.

Sample collection

Venous blood samples were taken from study participants in sterile vials. Routine investigations were completed on the same day. The serum for adiponectin, lipid profile, and thyroid hormones (T3, T4, and TSH), were then stored at −20°C until the serum was batch examined for various parameters.

The BMI of study participants was calculated by dividing their height in meters squared by their weight in kilograms: Weight (kg)/height squared (m^2) equals BMI.[10] Venipuncture samples were collected 12h after the individuals broke their fast. Centrifugation was used to separate the serum samples for 15 min at 3000 ×g. Serum thyroid stimulation levels were measured for the following parameters: lipid profile, adipolin, thyroxine (TT4), triiodothyronine (TT3), TSH, and enzyme-linked immune sorbent assay (ELISA) kit (Sunlong Medical Company, China). The colorimetric approach was utilized to generate quantifiable data. The levels of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL) were measured using BIOLABO kits (France). Friedewald's equation was used to calculate the low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) using the indirect technique.[11]

Statistical analysis

The variables were measured and reported as mean ± SD. The mean values of the two groups were compared using independent-sample *t*-tests (Student's *t*-test). The statistical

software SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used for the analyses. A statistically significant *P* value was determined to be less than 0.05. Pearson's correlation coefficient was used to evaluate the relationship between research factors. Using the MedCalc program, receiver operating characteristic (ROC) curves were generated to assess the usefulness of biomarkers for the diagnosis and prognosis of hypothyroidism. The area under the curve (AUC) was computed in order to assess the test's reliability. Differences with probabilities of less than 5% or 1% were deemed statistically significant, according to the selection of $P < 0.05$ and $P < 0.01$ for statistical significance.

Ethical approval

Each participant (both patients and controls) provided written informed consent through their parents, in accordance with the highest ethical standards. The institutional ethics committee of the University of Kufa approved the study on March 4, 2024, and all ethical and privacy standards (local, national, and international) were followed. These guidelines include the Belmont Report, the Council for International Organizations of Medical Sciences Guidelines, international conferences on harmonizing good clinical practice, and the World Medical Association's Helsinki Declaration. In addition, the guidelines for the safety of human research conducted by the International Council for Harmonization of Good Clinical Practice (ICH-GCP), are followed by our institutional review board.

Results

The study included 60 volunteers, divided into two groups: 60 individuals with hypothyroidism (30 SCH and 30 OH) and 30 healthy controls. Table 1 shows the demographics of the study participants. Compared

Table 1: The clinical features of individuals with and without hypothyroidism

Parameters	Control group No = 30 Mean ± SD	Patient groups (*n* = 60) Mean ± SD		>*P* value
		OH group No = 30 Mean ± SD	SCH group No = 30 Mean ± SD	
Sex (F/M)	**17/ 13**	**17/13**	**17/13**	—
Age (Years)	35 ± 10.56	36.2 ± 9.52	33.9 ± 5.71	0.246[a] 0.129[b] 0.239[c]
BMI (Kg/m^2)	22.83 ± 5.39	27.405 ± 3.802	25.66 ± 3.13	0.098[a] 0.023[b] 0.073[c]
TT3 (ng/mL)	1.902 ± 0.629	0.08 ± 0.07	0.26 ± 0.43	0.062[a] 0.079[b] 0.031[c]
TT4 (ng/mL)	73.82 ± 21.305	27.89 ± 9.68	37.33 ± 12.19	0.091[a] 0.057[b] 0.004[c]
TSH (µIU/mL)	1.83 ± 0.74	28.68 ± 15.56	6.67 ± 2.33	0.074[a] 0.053[b] 0.023[c]
T-CHO (mg/dL)	187.44 ± 25.624	198.15 ± 38.86	199.73 ± 24.03	0.002[a] 0.042[b] 0.059[c]
TG (mg/dL)	113.74 ± 32.03	171.46 ± 33.46	188.33 ± 32.36	0.000[a] 0.001[bb] 0.048[c]
HDL-C (mg/dL)	38.92 ± 7.342	23.71 ± 6.28	22.42 ± 7.47	0.001[a] 0.001[bbb] 0.438[c]
VLDL-C (mg/dL)	29.08 ± 7.541	34.28 ± 6.69	41.57 ± 13.33	0.004[a] 0.040[b] 0.348[c]
LDL-C (mg/dL)	117.66 ± 10.472	130.95 ± 22.88	135.57 ± 34.87	0.001[a] 0.001[b] 0.305[c]
Adipolin (pg/mL)	22.879 ± 2.219	9.292 ± 2.743	15.57 ± 2.692	0.071[a] 0.075[b] 0.021[c]

Table 2: Correlation between biochemical markers and blood adipolin levels in patients recruited in the SCH group

Parameters	*r*	*P* value	Parameters	*r*	*P* value
Age (Years)	−0.104	0.058	TG (mg/dL)	0.478	0.001
BMI (Kg/m^2)	−0.363	0.004	TC (mg/dL)	0.347	0.001
TT3 (ng/mL)	0.390	0.003	LDL-C (mg/dL)	0.333	0.001
TT4 (ng/mL)	0.628	0.001	VLDL-C (mg/dL)	0.348	0.001
TSH (ng/mL)	−0.810	0.001	HDL-C (mg/dL)	−0.259	0.031

r is the Pearson correlation coefficient. Body metabolic index (BMI), thyroid-stimulating hormone (TSH), thyroxine (TT4), triiodothyronine (TT3), total cholesterol (T-CHO), triglycerides (TG), HDL (high-density lipoprotein cholesterol), LDL (low-density lipoprotein cholesterol), and (VLDL-C:) very low-density lipoprotein cholesterol

Table 3: Correlation between serum adipolin levels and biochemical markers in recruited participants in the OH group

Parameters	r	*P* value	Parameters	r	*P* value
Age (Years)	−0.064	0.073	TG (mg/dL)	−0.101	0.449
BMI (Kg/m^2)	−0.102	0.058	TC (mg/dL)	−0.065	0.621
TT3 (ng/mL)	−0.168	0.037	LDL-C (mg/dL)	−0.001	0.992
TT4 (ng/mL)	0.287	0.012	VLDL-C (mg/dL)	−0.065	0.621
TSH (ng/mL)	−0.749	0.001	HDL-C (mg/dL)	0.269	0.037

r is the Pearson correlation coefficient. Body metabolic index (BMI), thyroid-stimulating hormone (TSH), thyroxine (TT4), triiodothyronine (TT3), total cholesterol (T-CHO), triglycerides (TG), HDL (high-density lipoprotein cholesterol), LDL (low-density lipoprotein cholesterol), and (VLDL-C:) very low-density lipoprotein cholesterol

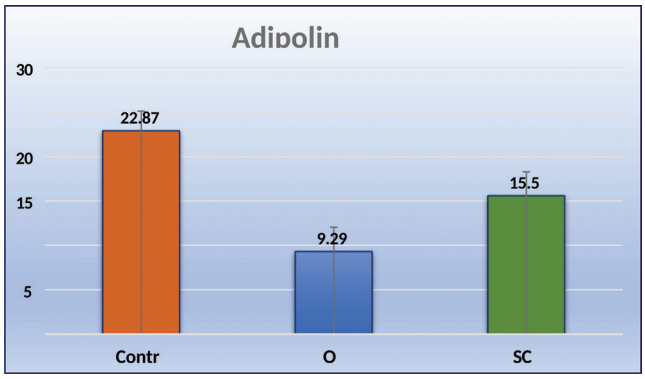

Figure 1: Graphical representation for comparison of serum adipolin between OH, SCH, and control group

to the healthy control group, the SCH study group was obese and had aberrant glucose and lipid profiles [Table 2].

Correlation analysis

According to Table 3, adipolin serum levels in SCH patients had a significant negative relationship with age, BMI, TSH, TC, VLDL-C, TG, and LDL-C. Adipolin significantly reduces serum levels of T3, T4, and HDL-C in the patient's group, as shown in [Figures 1–3 and Table 4].

In Table 5 and Figure 4, the statistical results of the adipolin biomarker's ROC analysis are presented. Adipolin's ROC curve shows an OH group cutoff value of ≤14.43, an AUC of 1.000, sensitivity and specificity of 100, and a *P* value of <0.001.

Discussion

The inability of the thyroid gland to produce adequate thyroid hormone is characteristic of hypothyroidism. A slow metabolism can cause weariness, weight gain, diarrhea, dry skin, and a decrease in overall body heat production. Hypothyroidism is more common among the elderly and affects women more than males. Nonetheless, it can occur at any age and can affect anyone at any time. HT, an autoimmune disease, is the most prevalent cause of hypothyroidism; however, there are other potential reasons as well, such as thyroid surgery, radiation therapy, and some drugs.[12]

Numerous nonspecific symptoms may indicate thyroid dysfunction and the diagnosis of this ailment is primarily dependent on the detection of abnormalities in biological pathways. The pituitary hormone thyrotropin (TSH), commonly known as thyrotropin, has a complex inverse relationship with the thyroid hormones thyroxin (T4) and triiodothyronine (T3).[13]

TSH levels are the most sensitive indicator of an individual's thyroid health because of the negative feedback loop between TSH and thyroid hormones.[14]

OH is diagnosed in a person with low free T4 levels and elevated TSH levels. When circulating T4 levels are normal but TSH levels are elevated, it is regarded as hypothyroidism that is subclinical.[15]

An elevated risk of coronary artery disease has been associated with hypothyroidism, whether it be overt or subclinical. Reduced thyroid hormone levels are believed

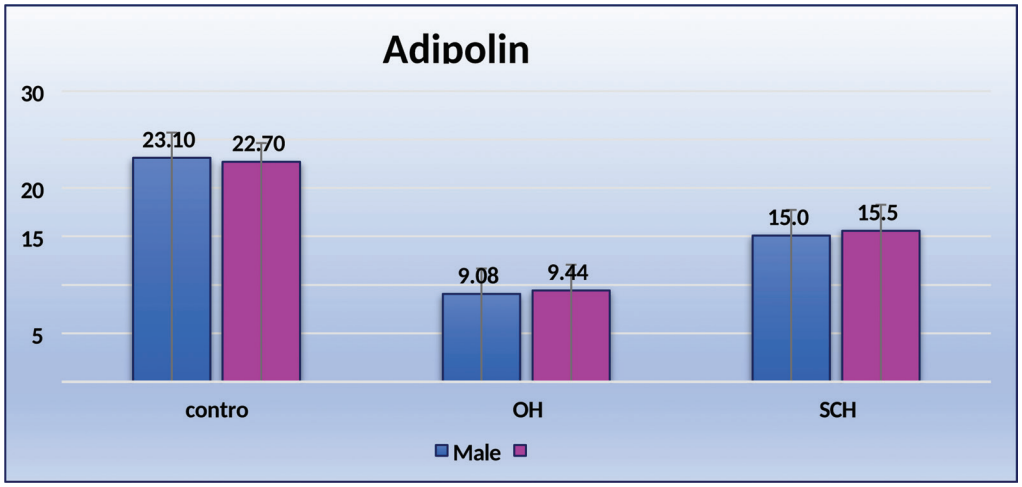

Figure 2: Graphical representation for comparison of serum adipolin between females and males SCH, OH, and control groups

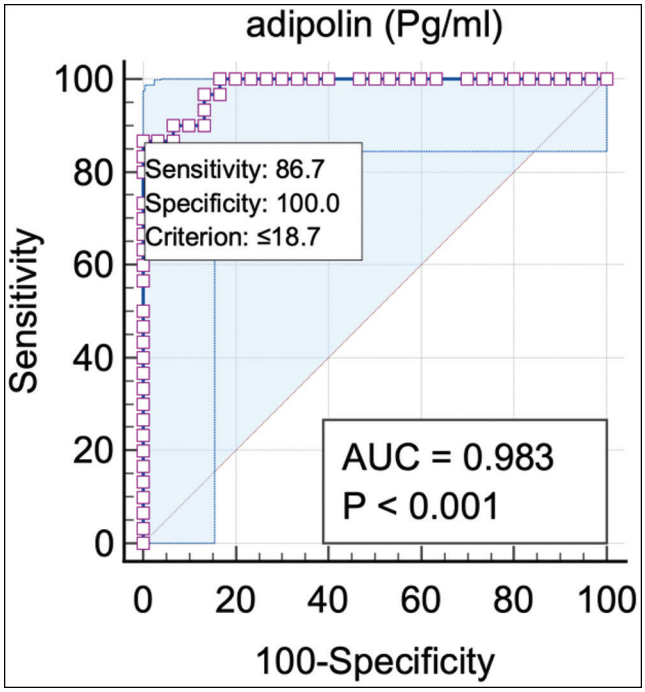

Figure 3: The ROC curve for adipolin compares SCH patients and healthy controls

Table 4: Statistics output of ROC analysis of markers adipolin between healthy and subclinical hypothyroidism patients

Variables	Adipolin (pg/mL)
Cutoff value	≤18.7
Sensitivity	86.7
Specificity	100.0
Area Under Curve C)	0.983
P value	<0.001
Accuracy (Youden Index)	0.8667
Standard Error [a]	0.0111
95% Confidence interval [b]	0.911 to 1.000

Table 5: Output statistics for ROC analysis of indicators between healthy and patients with overt hypothyroidism

Variables	Adipolin (Pg/ml)
Cutoff value	≤14.43
Specificity	100
Sensitivity	100
P value	<0.001
Area Under Curve (AUC)	1.000
Accuracy (Youden Index)	1.0000
Standard Error[a]	0.000
95% Confidence interval[b]	0.940 to 1.000

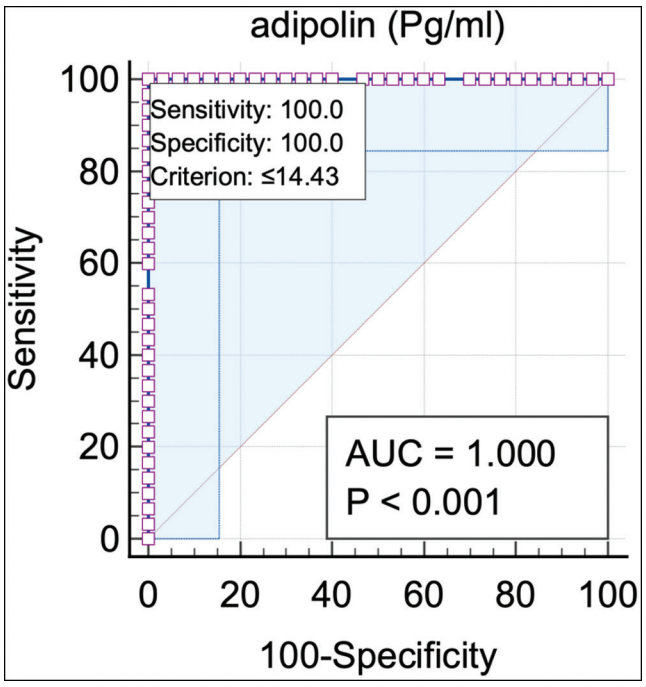

Figure 4: The ROC curve for adipolin compares OH patients and healthy controls

to have an impact on lipid metabolism and vascular health.[16]

Additionally, the inflammatory proteins of the adipose tissue enhance insulin resistance. If cultured macrophages are supplied with the culture medium of adipolin-expressing cells, it prevents the inflammatory-induced pro-inflammatory mediators. It is therefore possible to use adipolin to improve insulin resistance because it improves insulin sensitivity and has anti-inflammatory properties.

A prior study demonstrated showed the absorption of insulin-stimulated glucose in adipose tissue can only be increased by full-length fCTRP12.

An important correlation exists between adipolin and type 2 diabetes (T2DM) as well as improved insulin and glucose tolerance in people with diet-induced obesity.[17]

Adipolin decreases inflammation in adipose tissue, hence enhancing insulin sensitivity in obese rats. Adipolin also increases glucose metabolism via insulin-dependent and independent mechanisms. Furthermore, adipolin has been found to limit the proliferation of smooth muscle cells and the inflammatory response of macrophages, thereby attenuating the aberrant vascular remodeling following artery injury.[18]

Adipokine imbalance, the majority of which are pro-inflammatory and metabolically harmful, is thought to contribute to the development of obesity-related metabolic problems in hypothyroidism. Adipolin levels in the blood have an inverse relationship with various disorders, such as T2DM and other conditions associated with fat (1, 3, 10). According to this study, fat reduces the levels of adipolin in both adipose tissue and blood, and adipolin is mostly expressed in adipose tissue. Comparable to adipolin, it suppresses inflammatory reactions in malnourished WAT, which could enhance glucose metabolism.[19]

Conclusion

The concentration of adipolin was significantly lower in people with OH and SCH compared to healthy controls. Adipolin may be an independent prognostic marker for metabolic problems and a risk factor for this disease.

Acknowledgment

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Conflicts of interest

There is no conflict of interest, according to the authors.

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